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VLIV BIODEGRADABILNÍCH POLYMERŮ NA PŮDNÍ VLASTNOSTI A ROSTLINNOU BIOMASU

EFFECTS OF BIODEGRADABLE POLYMERS ON SOIL PROPERTIES AND PLANT BIOMASS

DIZERTAČNÍ PRÁCE

DOCTORAL THESIS

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Zadání dizertační práce

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Název dizertační práce:

Vliv biodegradabilních polymerů na půdní vlastnosti a rostlinnou biomasu

Zadání dizertační práce:

Zpracování literární rešerše na téma vlivu konvenčních a biodegradovatelných mikroplastů na půdu a růst rostlin.

Testování vlivu mikrobioplastů vybraného polyhydroxyalkanoátu na vlastnosti půdy s přihlédnutím k půdní mikrobiologii a textuře půdy.

Testování vlivu biodegradace mikrobioplastů vybraného polyhydroxyalkanoátu na růst rostlin a dynamiku živin.

Testování možností potlačení negativních důsledků živinové nerovnováhy.

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Dizertační práce se odevzdává v děkanem stanoveném počtu exemplářů na sekretariát ústavu. Toto zadání je součástí dizertační práce.

Ing. Martin Brtnický Student	prof. Ing. Jiří Kučerík, Ph.D. Vedoucí práce	prof. Ing. Jozef Krajčovič, Ph.D. Ředitel ústavu

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Děkan

OBSAH

Abstrakt

Biodegradabilní plasty jsou stále častěji využívány jako náhrada konvenčních, biologicky nerozložitelných, plastů. Nicméně, zatímco vliv konvenčních plastů a jejich fragmentů na životní prostředí je již rozsáhle studován, výzkum týkající se vlivu biodegradabilních plastů na půdu je zatím na počátku. První studie v této oblasti potvrdily opodstatněnou obavu týkající se možného vlivu biodegradace na rovnováhu půdních živin, nicméně detailní studie mechanismů, účinků na půdní mikrobiom, a především možných řešení doposud chyběly.

Tato práce si klade za cíl tyto mezery alespoň částečně zaplnit. Z tohoto důvodu byla provedena série experimentů studujících vliv biodegradace poly-3-hydroxybutyrátu (P3HB) na půdy s variabilními vlastnostmi a růst rostlin. Studovány byly především vlivy na změnu aktivity půdního mikrobiomu, kvalitu půdní organické hmoty, tok živin, nárůst a kvalitu rostlinné biomasy a míru/ rychlost degradace P3HB. Byly také testovány možné přístupy pro potlačení negativního vlivu P3HB na růst rostlin. Výsledky prokázaly, že biodegradace P3HB způsobuje nárůst mikrobiální aktivity a určitý posun v biodiverzitě, nerovnováhu a změny v toku živin vytvářejících stres, který negativně ovlivnil růst rostlin. Jako možné snížení stresu se ukázala aplikace digestátu, který zmírnil negativní vliv biodegradace P3HB na růst rostlin.

Biodegradabilní plasty, včetně P3HB, představují jednu z možností řešení plastového znečištění, nicméně, ani jejich vliv na životní prostředí není benigní. Proto je důležité důkladně pochopit jejich vliv na životní prostředí a předcházet tak potencionálním rizikům při jejich použití i likvidaci.

Klíčová slova

Poly-3-hydroxybutyrát, půdní mikrobiom, půdní enzymatická aktivita, DNA, biodegradace, půdní organická hmota, environmentální stres.

Abstract

Biodegradable plastics are increasingly being used as alternatives to conventional, non-biodegradable plastics. However, while the environmental impacts of conventional plastics and their fragments have been extensively studied, research on the effects of biodegradable plastics on soil is still in its early stages. Initial studies in this field have confirmed justified concerns regarding the potential impact of biodegradation on soil nutrient balance. However, detailed studies into the mechanisms, effects on the soil microbiome, and especially potential mitigation strategies have so far been lacking.

This study aims to partially fill these gaps. A series of of experiments was conducted to investigate the effects of poly-3-hydroxybutyrate (P3HB) biodegradation on soils with varying properties and on plant growth. The research focused primarily on changes in soil microbial activity, soil organic matter quality, nutrient fluxes, plant biomass growth and quality, and the extend and rate of P3HB degradation. Potential approaches to mitigate the negative impact of P3HB on plant growth were also tested. The results demonstrated that P3HB biodegradation lead to increased microbial aktivity, shifts in mirobial biodiversity, imbalances and changes in nutrient fluxes, ultimately creating stress conditions that negatively affected plant growth. Digestate application was idnetified as a promising stratégy to mitigate the negative impacts of P3HB degradation on plant development.

Although biodegradable plastics, including P3HB, represent a potential solution to plastic pollution, their environmental impact is not entirely benign. Therefore, it is essential to thoroughly understand their interactions with the environment to anticipate and prevent potential risks associated with their use and disposal.

Key words

Poly-3-hydroxybutyrate, soil microbiome, soil enzyme activity, DNA, biodegradation, soil organic matter, environmental stress.

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Prohlášení

Prohlašuji, že jsem disertační práci vypracoval samostatně a že všechny použité literární zdroje jsem správně a úplně citoval. Disertační práce je z hlediska obsahu majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího práce a děkana FCH VUT.

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Ing. Martin Brtnický

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Na tomto místě bych rád poděkoval mému vedoucímu prof. Ing. Jiřímu Kučeríkovi, Ph.D. za možnost studovat, dlouhé diskuse, cenné rady, přátelský přístup, trpělivost a vedení při celém studiu a psaní této disertační práce. Mé velké poděkování za přínosné poznámky, pomoc a podporu patří i kolegům Vaškovi Pecinovi a Jirkovi Holátkovi. Edovi, že mě vedl při prvních výzkumných krůčcích týkajících se půdy. Elišce Kameníkové za to, že mě vždy navedla ke správným dveřím. Taktéž nesmím zapomenout poděkovat ostatním kolegům a to jak za pomoc, tak i směřování k odevzdání této práce. V neposlední řadě bych chtěl poděkovat mé rodině, především pak mé manželce a dětem za podporu a vytvoření podmínek potřebných k dokončení této práce, stejně tak jako svým blízkým přátelům.

SEZNAM PŘÍLOH

Příloha A

Dose-dependent effects of poly-3-hydroxybutyrate on soil quality and maize development: A trade-off between soil quality and crop productivity.

Příloha B

Effect of stabilized organic amendments on biodegradability of poly-3-hydroxybutyrate, soil biological properties, and plant biomass.

Příloha C

Biodegradation of poly-3-hydroxybutyrate after soil inoculation with microbial consortium: Soil microbiome and plant responses to the changed environment.

Příloha D

Effect of biodegradable poly-3-hydroxybutyrate amendment on the soil biochemical properties and fertility under varying sand loads.

Příloha E

Soil texture-driven modulation of poly-3-hydroxybutyrate (P3HB) biodegradation: Microbial shifts, and trade-offs between nutrient availability and lettuce growth.

Příloha F

Biodegradable microplastics impact on soil: How poly-3-hydroxybutyrate alters microbial diversity and nitrogen mineralization processes.

1 ÚVOD

Termín plasty se běžně používá pro označení široké škály syntetických nebo polosyntetických materiálů, které se používají v obrovském a stále rostoucím množství aplikací. Od zahájení masové výroby ve 40. letech 20. století se plasty staly všudypřítomnou součástí lidského života. V roce 2019 se celosvětová produkce plastů zvýšila na téměř 368 milionů tun. Očekává se, že do roku 2050 se výroba plastů ztrojnásobí a bude představovat pětinu celosvětové spotřeby ropy (www.statista.com). Se zvýšenou produkcí a spotřebou plastu je nezbytně spojena i zvýšená kontaminace životního prostředí a dále pak tvorba plastových fragmentů, mikro a nanoplastů (MP a NP) – téma, které rezonuje společností, a které je i přes rostoucí počet vědeckých prací stále ještě prozkoumáno velmi povrchně.

Předložená disertační práce se zaměřuje na biodegradabilní plasty, které bezesporu představují jedno z možných řešení plastové kontaminace. Nicméně, jak naznačují výsledky nedávných studií, jejich vliv na životní prostředí nemusí být úplně benigní, jak je některými jejich propagátory naznačováno. Dojde-li v budoucnu ke snížení ceny biodegradabilních plastů a tím i k podpoře jejich masivního využití, je nutné dostatečně dopředu prozkoumat i jejich případná negativa, aby se společnost vyhnula případným environmentálním problémům.

Tato práce se tematicky zaměřuje na důležitou část tohoto problému, konkrétně na vliv biodegradabilních plastů na produkční a mimoprodukční funkce půdy. Disertační práce je organizována do několika kapitol. V teoretické části se zaměřuje na plasty v zemědělství, je popisován vliv MP a mikrobioplastů na fyzikální, chemické a mikrobiologické vlastnosti půdy a na růst rostlin. V této části práce jsou brány v potaz vlivy jak nebiodegradabilních, tak i biodegradabilních plastů. Důvodů je několik, mezi nejdůležitější patří především i) nejasná hranice oddělující biodegradabilní a nebiodegradabilní plasty, ii) prolínání biotických a abiotických faktorů vedoucích k tvorbě MP v reálných podmínkách, iii) podobnost vlivu na životní prostředí, přinejmenším v počátečních stádiích kontaminace a iv) podobnost cest, jakým ke kontaminaci životního prostředí dochází. V další části jsou pak na základě literární rešerše definovány cíle disertační práce a výzkumné otázky. V poslední části jsou pak uvedena a diskutována některá zjištění s odkazem na autorem publikované práce a na práci v recenzním řízení. Všechny relevantní publikace autora jsou pak součástí poslední části práce ve formě příloh.

2 TEORETICKÁ ČÁST

2.1 Plasty v zemědělství

V zemědělství je používána celá škála plastů s různými mechanickými a fyzikálně-chemickými vlastnostmi. Mezi nejčastější patří vysokohustotní polyethylen (HDPE), který nachází uplatnění jako materiál pro výrobu zemědělských sítí (Castellano et al. 2008) a mulčovacích fólií (Steinmetz et al. 2016), stejně tak jako lineární nízkohustotní polyethylen (LLDPE) (Espí et al. 2016). Polypropylen (PP) je využívaný pro výrobu netkaných textilií (Castellano et al. 2008) a společně s polyvinylchloridem (PVC) pak jako materiál pro zavlažovací trubky nebo hadice (Scarascia-Mugnozza et al. 2012). PVC, polyolefiny, ethylen-vinylacetát (EVA) nebo kopolymery ethylen-butyl-akrylátu (EBA) se také používají jako kryty skleníků (včetně speciálních typů, např. fólie blokující blízké infračervené záření (NIR) s kovovými pigmenty, ultratermické fólie, průchozí tunely a kryty nízkých tunelů (Espí et al. 2016; Maraveas 2019). Polykarbonát (PC) a polymetylmetakrylát (PMMA) jsou často využívány jako dvouvrstvé (alveolární) tepelně izolační kryty skleníků (Scarascia-Mugnozza et al. 2012). Polystyren (PS) a polyuretan byly v minulosti hojně používány pro tzv. coating hnojiv (Yang et al. 2012; Qu et al. 2019a). Tento seznam není kompletní, ale ilustruje široké využití plastů v zemědělství a příbuzných oborech, a tedy i potencionální zdroje kontaminace půdy (a dalších složek životního prostředí) plastovými fragmenty (Jansen et al. 2019).

2.2 Produkce MP

Plastové materiály používané v zemědělství jsou běžně vystaveny mnoha vlivům, a to jak vlivům prostředí (např. slunečnímu záření, vlhkosti, mikrobiálnímu napadení atd.), tak i vlivům souvisejícím s jejich fungováním (především mechanickému namáhání). Toto namáhání vede k postupnému poškozování plastů a jejich degradaci. Kinetika degradace pak závisí na tloušťce plastu, mechanických a optických vlastnostech, teplotě a relativní vlhkosti (a jejich fluktuaci), větrné a dešťové erozi ale také způsobu instalace. Sluneční záření, především pak jeho vysokoenergetická ultrafialová (UV-B; 290–315 nm) a středně energetická (UV-A; 315–400 nm) složka záření iniciuje vznik volných radikálů, které jsou hlavní příčinou fotodegradace plastů (Liu et al. 2019). Degradace ultrafialovým zářením může být zintenzivněna současným mechanickým otěrem (Tian et al. 2022). K mechanické degradaci může také vést zmrznutí a rozmražení plastů za spolupůsobení půdní vlhkosti (Pal et al. 2018). Rozpad plastů na menší části společně s větrem (Zalasiewicz et al. 2016) způsobuje šíření plastových fragmentů do vzdálenějších oblastí (Allen et al. 2019).

Plastové fragmenty nazývané plastový odpad se obecně dělí podle velikosti na megaodpad (>100 mm), makroodpad (100–20 mm), mezoodpad (20–5 mm) a mikroodpad (<5 mm) (Barnes et al. 2009). Všechny tyto velikosti jsou některými autory souhrnně označovány jako MP, tedy označením, kterým byly v roce 2004 prvotně popisovány malé plastové částice v oceánech. Z důvodu nejednoznačného členění další autoři navrhují i jinou kategorizaci (Pinto da Costa 2018) – jsou definovány i kategorie pro menší částice (NP – a to buď pod 1 nebo 0.1 μm (Koelmans et al. 2015; Mattsson et al. 2015). Nicméně, tato problematika je mimo zaměření této práce. Podstatné je, že MP byly nalezeny celosvětově v mnoha různých prostředích, včetně lidského těla (Thompson et al. 2004) a placenty novorozenců (Ragusa et al. 2021), což podtrhuje fakt, že MP jsou všudypřítomné a prioritizuje výzkum jejich efektů na složky životního prostředí.

V zemědělské půdě lze nalézt dvě skupiny MP: primární MP, které byly vyrobeny záměrně a použity v kosmetických výrobcích a různých průmyslových odvětvích, a které se do půdy dostaly například po aplikaci čistírenského kalu. Dále pak sekundární MP, které jsou degradačními produkty větších plastových výrobků nebo odpadů. Ty se dostávají do půdy například rozpadem mulčovacích folií. V současné době jsou v životním prostředí všudypřítomné jak primární, tak sekundární MP.

Zdrojem většiny plastů v akvatických systémech jsou aktivity probíhající na souši. Znečištění zahrnuje plasty všech velikostí včetně MP (Zubris a Richards 2005; Rillig 2012). Množství plastového odpadu, které se dostává do půdy, je vysoké, například v Evropě se ke zpracování (např. recyklaci) dostalo v roce 2014 pouze zhruba 25,8 milionu tun, což odpovídá 54 % ročně vyprodukovaného plastového odpadu (de Souza Machado et al. 2018a). Odhaduje se, že celosvětově může zhruba 32 % plastového odpadu skončit v půdě nebo kontinentálních vodních ekosystémech (Jambeck et al. 2015). Dále pak, práce autorů (de Souza Machado et al. 2018a) poukázala na to, že na pevnině může být 4 až 23krát více MP ve srovnání s oceánem (Horton et al. 2017). Orná půda může obsahovat více MP než slaná voda; v Evropě by se mohlo do zemědělské půdy ročně dostat 63–430 kilotun MP, v Severní Americe pak 44–300 kilotun MP (Nizzetto et al. 2016b). Stále však bohužel platí, že studie založené na spolehlivých kvantifikačních metodách (např. Choi et al. 2020) jsou bohužel stále vzácné, nekonzistentní a datově nehomogenní.

2.3 Hlavní vstupy plastů do zemědělských půd

Jak již bylo naznačeno v předcházejících kapitolách, hlavní vstupy plastů do zemědělských půd zahrnují:

i. časté používání plastových materiálů a zařízení, jakými jsou mulčovací fólie, sítě a nádrže, hadice zavlažovacích systémů, kompozity minerálních hnojiv a bioplastů, coating hnojiv atd. (Castellano et al. 2008; Espí et al. 2016; Steinmetz et al. 2016; Maraveas 2019);
ii. hnojení zemědělskými kaly, komposty, statkovým a drůbežím hnojem, které jsou (navzdory metodám třídění a screeningu před kompostováním i po něm) stále kontaminovány MP v koncentracích od 2,38–180 mg/kg (Blasing a Amelung 2018); jiné zdroje pak uvádí 1,2 g/kg (Gao et al. 2019a);
iii. hnojení čistírenskými kaly (aplikovanými bez kompostování/s kompostováním, obsahujícími MP filtrované a koncentrované v čistírnách odpadních vod) (Nizzetto et al. 2016b; Willen et al. 2017; Ng et al. 2018; Khalid et al. 2020; Milojevic a Cydzik-Kwiatkowska 2021);
iv. zavlažování recyklovanou odpadní vodou nebo sladkou vodou (kontaminovanou MP z komunálního odpadu) (Corcoran et al. 2010; Mintenig et al. 2017) – MP byly nalezeny jak v evropských (Sadri a Thompson 2014), tak i severoamerických (Zbyszewski et al. 2014) a asijských (Free et al. 2014) povrchových vodách;
v. atmosférická depozice (expozice může dosahovat až 280 ks/m^2 MP za den) (Dris et al. 2016), přičemž důležitým zdrojem je například silniční doprava (Baensch-Baltruschat et al. 2021); a
vi. méně významné, ale nikoliv zanedbatelné, jsou zdroje, jakými jsou odpadky (littering) a pouliční splachy (Blasing a Amelung 2018).

V praxi jsou plastové odpady ponechávány na polích nebo podél vodních toků, spalovány na volném prostranství, zakopávány do půdy (Zalasiewicz et al. 2016) nebo ukládány na skládky, odkud se při nesprávném uložení mohou šířit do životního prostředí (Scalenghe 2018). Nevhodná likvidace zemědělského plastového odpadu způsobuje kontaminaci půdy (He et al. 2019) a vody, těkavé látky se uvolňují do ovzduší (Horton a Dixon 2018) a všechny tyto zdroje znečištění pak mohou mít za následek kontaminaci potravin (Bouwmeester et al. 2015), zhoršování kvality půdy a agroekosystému a estetické znečištění (Kyrikou a Briassoulis 2007) a také zhoršování stavu krajiny (Briassoulis et al. 2013). Díky tomu začíná být pro zemědělskou praxi doporučováno používání biodegradabilních plastových materiálů (Vox et al. 2016).

Jak vyplývá z výše uvedeného výčtu, mezi hlavní zdroje primárních MP patří čistírenské kaly, které obsahují MP z produktů osobní hygieny nebo pro domácnost (Zubris a Richards 2005), včetně polyesteru a nylonu, hlavních polymerů používaných v syntetických textiliích, a mikroperliček a třpytek v kosmetice na bázi polyethylenu (PE) nebo PP. Následkem toho bylo v půdách se známou historií aplikace čistírenských kalů nalezeno výrazně vyšší množství právě syntetických mikrovláken než v půdách, kde se kaly neaplikovaly (Zubris a Richards 2005). Syntetická mikrovlákna byla na některých polích nalezena i 15 let po poslední aplikaci kalů (Zubris a Richards 2005).

Současné předpisy týkající se škodlivých látek v kalech aplikovaných na půdu nepovažují MP za hrozbu (byť se situace mění), takže množství MP, které se ročně neúmyslně dostanou do půdy, může být vyšší než je odhadováno (Nizzetto et al. 2016a).

Hlavní zdroj sekundárních MP pak představuje mulčování, které se používá k ochraně sazenic a výhonů prostřednictvím izolace, udržování půdního mikroklimatu (Kasirajan a Ngouajio 2012), teploty (Ham et al. 1993), zabránění odpařování půdní vody (Kader et al. 2017), úpravy propustnosti nebo odrazivosti specifických vlnových délek dopadajícího slunečního záření, úpravy kořenové zóny (Tarara 2000; Ibarra-Jimenez et al. 2011) a regulaci výměny plynů (Diaz-Perez 2010; Torres-Olivar et al. 2018). Současné předpisy v různých zemích se snaží problém produkce MP z mulčování řešit, např. v Evropské unii je povoleno nahradit mulčovací fólie z konvenčních plastů biologicky rozložitelnými plasty (EN_17033 2018). Tato změna, jak je diskutováno dále, však nemůže „MP problém“ uspokojivě vyřešit.

2.4 Transformace plastů v půdě

Pokud se (mikro)plasty z různých zdrojů a vektorů (půdní změny, voda, rozklad in situ) dostanou do půdy, je jejich následná perzistence významně ovlivněna půdním potenciálem k rozkladu a chemickým složením plastového materiálu. O rozkladu (mikro)plastů v půdě se zmiňuje jen málo výzkumů (ve srovnání se studiemi o rozložitelnosti plastů ve vodním prostředí) (Binda et al. 2024, Nikolic et al. 2014; Osman et al. 2017; Roy et al. 2021).

(Mikro)plasty jsou obecně kategorizovány podle přítomnosti nebo nepřítomnosti esterových nebo amidových skupin na hydrolyzovatelné nebo nehydrolyzovatelné. Tyto vlastnosti a kategorizace určují jejich dostupnost pro různé extracelulární hydrolázy. Podle (Singh a Sharma 2008; Ng et al. 2018) je pravidlem, že biologicky odbouratelné MP mají ve své struktuře heteroatomy kyslíku (O), dusíku (N) a síry (S). Tyto atomy, nebo přesněji vazby mezi těmito atomy a uhlíkem (C), jsou cílem pro hydrolytické enzymatické působení půdní mikroflóry. Samotný proces biodegradace je pak rozkladem sloučenin (plastů) na jednodušší molekuly, vedoucí nakonec až k mineralizaci mikroorganismy za vzniku CO_2 a H_2O (aerobní) nebo CO_2 a CH_4 (anaerobní) (Ng et al. 2018; Ghosh et al. 2019). Ve skutečnosti je anaerobní biodegradace plastů ve srovnání s aerobní degradací energeticky méně výhodná a trvá delší dobu, než dojde k úplné mineralizaci (Gu 2003).

Přestože většina plastů používaných v zemědělství je údajně vysoce odolná vůči přirozené depolymerizaci a mikrobiálnímu využití, některé studie naznačují opak. Příkladem je polyethylentereftalát (PET), který představuje důležitý materiál v obalovém průmyslu. Podle některých zdrojů (Qi et al. 2021) vyžaduje biodegradace PET mírnější teploty a nižší spotřebu energie než recyklační metody, což ji činí použitelnou a slibnou biorecyklační strategií. Bakterie *Ideonella sakaiensis*, která byla nalezena (a patrně se zde vyvinula) nedaleko recyklačního závodu v Japonsku, disponuje PET-ázou, tj. enzymem schopným hydrolyzovat PET (Danso et al. 2018). Další enzymy, jako je kutináza, lipáza, serinesteráza a nitro-benzyl-esteráza, jsou rovněž schopny této hydrolýzy, zatímco například proteáza, kutináza, amidáza a hydroláza hydrolyzují také polyamid (PA) (Guebitz a Cavaco-Paulo 2008).

Enzymatická hydrolytická a oxidační degradace plastů vede ke štěpení makromolekuly polymeru za vzniku polymerů s krátkým řetězcem a malých molekulárních fragmentů (např. oligomerů, dimerů a monomerů). Molekulová hmotnost těchto degradačních produktů je pak dostatečně malá na to, aby mohly projít buněčnou stěnou (Chen et al. 2019) a po asimilaci mikroorganismy pak podléhat intracelulární degradaci (Wilkes a Aristilde 2017).

2.5 Biologicky rozložitelné plasty

Chemické složení plastů determinuje výskyt a vznik MP, jejich přenos, perzistenci, rozložitelnost a potenciální škodlivé účinky. Z těchto důvodů se do centra pozornosti dostávají biologicky odbouratelné plasty, které, jak již bylo zmíněno v úvodu, mohou hrát zásadní roli při řešení problémů s likvidací plastů z ekonomického i environmentálního hlediska (Thakur et al., 2018).

Biologicky rozložitelné (nebo též biodegradabilní) plasty jsou obvykle směsi biologicky rozložitelných polymerů s různými přísadami. Lze je rozdělit do tří hlavních skupin: (i) přírodní polymery (škrob, celulóza a lignin), (ii) polymery z biomasy, buď syntetizované, nebo vzniklé při fermentaci (kyselina polymléčná – PLA; polyhydroxyalkanoát – PHA); a (iii) polymery syntetizované z fosilních zdrojů, jako polybutylen adipát-ko-tereftalát (PBAT), poly(ε-kaprolakton) (PCL), polybutylensukcinát (PBS) a polykarbonát (PC) (Ng et al. 2018; Gioia et al. 2021). Biodegradabilní plasty se vyrábějí převážně z obnovitelných zdrojů a mohou být rozkládány a metabolizovány celou škálou organismů včetně bakterií a hub (Kale et al., 2015).

Mezi biologicky rozložitelné polymery používané v zemědělství patří například výše zmíněné PLA, PCL, PBS, PBAT, PHA, stejně jako celulózy a škrob (Ng et al. 2018; Mo et al. 2023). Biodegradabilní plasty se zpravidla používají jako zemědělský mulč, často také jako obalový materiál. Mezi další aplikace patří trubky pro kapkovou závlahu a stíněné skleníkové tunely a materiály s prodlouženým uvolňováním pesticidů a hnojiv (Mo et al. 2023). Protože biologicky rozložitelné plasty mají obecně horší mechanické (tuhost, houževnatost) a tepelné vlastnosti, jsou buď míseny s jinými polymery (např. PLA a PBAT) (Colnik et al. 2020), nebo s přísadami, jakými jsou stabilizátory, změkčovadla, antioxidanty, oxidační promotory, povrchově aktivní látky prodlužující řetězce, kompatibilizátory a další (Cui et al. 2021).

Jak již bylo naznačeno, biodegradace v půdě probíhá v několika krocích a její rychlost závisí na podmínkách prostředí, jako je teplota, koncentrace živin, zda jsou přítomné organismy schopné biodegradace, vlhkost a obsah a kvality půdní organická hmoty. Kromě toho hraje důležitou roli také velikost a tvar biologicky rozložitelného materiálu a přísady použité pro zlepšení mechanických vlastností. Nicméně, vzhledem k rychlosti různých fází degradace, biologicky rozložitelné plasty mohu vytvářet tzv. mikrobioplasty (MBP), analogické částice k MP, a to dokonce mnohem rychleji v porovnání s MP (Sintim et al. 2023).

Jako příklad lze uvést studii autorského kolektivu (Li et al. 2023), kteří pozorovali, že MP PBAT/PLA vytvářely v půdním mikrobiálním společenstvu sítě s menší složitostí a více kompetitivními interakcemi než částice PE. To bylo vysvětleno jako důsledek toho, že mikročástice PLA/PBAT mají na svém povrchu více mikroskopických anomálií a více kontaktních míst pro tvorbu mikrobiálního biofilmu než částice, které nejsou biologicky rozložitelné. Kromě toho, využití polymerních sloučenin biologicky rozložitelných plastů zvyšuje množství dostupného C v půdě a snižuje množství dostupného N (Zhou et al. 2021a). To vedlo ke změně enzymatické aktivity půdního mikrobiomu v důsledku adaptace společenstva na tento substrát (Zhou et al. 2021a). Ve srovnání s rozsahem a variabilitou bakteriálních druhů schopných degradovat různé typy konvenčních organo-polymerních materiálů je početnost a rozšíření různých taxonů s metabolickými nástroji pro katabolismus biodegradabilních plastů větší (Awasthi et al. 2022). Důvodem je skutečnost, že řada biodegradabilních polymerů, jako jsou PLA, PHA a (samozřejmě) plasty na bázi celulózy, škrobu, ligninu, je rovněž na biologické bázi a mikrobiálně biosyntetizována.

Ne všechny bioplasty však lze považovat za biologicky odbouratelné a stejně tak ne všechny biologicky odbouratelné polymery jsou biosyntetizovány (Awasthi et al. 2022). Například PBAT vyrobený z petrochemických surovin je degradován houbami *Candida antarctica* (Xu et al. 2023) a různými bakteriálními kmeny (Thumarat et al. 2012; Bubpachat et al. 2018), *Saccharomonospora viridis* (Kawai et al. 2014) a termofilním kmenem *Thermomonospora fusca* z kompostu (Witt et al. 2001), které patří mezi aktinobakterie. Dále pak *Bacillus sp.* JY35 (Cho et al. 2022) a také zástupci rodů *Clostridium, Streptococcus* a *Caldicoprobacter*, nalezení v termofilních reaktorech pro rozklad směsi PLA/PBAT (Tseng et al. 2020). Vedle chemické syntézy se PLA biosyntetizuje také pomocí metabolicky upravené *Escherichia coli* (Jung et al. 2010), pomocí lipázy izolované z *Candida rugosa* (Whulanza et al. 2018) nebo pomocí *Yarrowia lipolytica* (Lajus et al. 2020). Velké množství mikroorganismů vykazuje schopnosti rozkladu PLA, např. bakterie z rodu *Actinobacteri*a, rody *Geobacillus* a *Bacillus* (Tomita et al. 2003), druhy *Stenotrophomonas pavanii* a *Pseudomonas geniculate* (Bubpachat et al. 2018), anaerobní *Tepidimicrobium xylanilyticum* (Tseng et al. 2020), houbové rody *Amycolatopsis* (Ikura a Kudo 1999), *Aspergillus* (Maeda et al. 2005), *Fusarium* (Torres et al. 1996), *Penicillium* (Jeszeova et al. 2018). Polykaprolakton (PCL) byl popsán jako biodegradovatelný lipázami *Candida antarctica* (Ma et al. 2020), *Pseudomonas cepacia* (Sivalingam et al. 2003), *Aspergillus fumigatus* (Hakkarainen a Albertsson 2002), *Fusarium* (Abe et al. 2010; Jeszeova et al. 2018). Polybutylensukcinát (PBS), syntetizovaný polykondenzací kyseliny jantarové a butandiolu, je degradován kutinázami z *Roseateles depolymerans* TB-87 a *Pseudozyma antarctica* JCM 10317 (Shinozaki et al. 2013), *Pseudomonas cepacia* (Taniguchi et al. 2002), dále *Thermobifida alba, T. cellulosilytica, T. fusca*, *Thermomonospora curvata* (Gamerith et al. 2017; Pan et al. 2018), *Aspergillus fumigatus* (Jung et al. 2018), *Fusarium solani* (Kitamoto et al. 2011) a *Pichia pastoris* (Peñas 2023). Polykarbonáty (PC), které se hojně vyskytují např. na skládkách, jsou biologicky rozložitelné mnoha bakteriemi, jako jsou *Pseudoxanthomonas sp. NyZ600* (Yue et al. 2021), *Bacillus cereus* a *B. megaterium* (Arefian et al. 2020), rody *Arthrobacter, Enterobacter* (Goel et al. 2008), *Duganella*, *Psuedomonas*, *Ralstonia*, *Roseateles*, *Variovorax*, *Acinetobacter* (Artham a Doble 2008) a houbami rodů *Fusarium, Ulocladium, Chrysosporium, Penicillium, Rhizopu*s (Arefian et al. 2013). PHA jsou striktně bakteriálně produkované polymerní plasty (Behera et al. 2022), proto enzymy pro jejich degradaci produkují především mikrobi schopní PHA biosyntetizovat. Nicméně, PHA degraduje i mnoho dalších mikroorganismů – rody *Pseudomonas* (Mohanan et al. 2020; Manoli et al. 2022), *Thermobifida sp.* (Phithakrotchanakoon et al. 2009), *Variovorax, Alcaligenes faecalis* (Sun et al. 2015), *Streptomyces exfoliatus* (Martinez et al. 2015), *Bacillus megaterium* (Chen et al. 2009). Degradaci PHA zvládají i četné houbové taxony, např. rod *Penicillium* (degradují také PLA) (Gowda a Shivakumar 2015; Jeszeova et al. 2018), nebo zástupci rodu *Fusarium*, kteří jsou schopni degradovat i PVA (polyvinylalkohol) (Abe et al. 2010; Jeszeova et al. 2018).

2.6 Vliv MP a MBP na půdu a biotu

Osud plastů v půdě a jejich vliv na půdní prostředí je v podstatě stále na začátku, znečištění půdního ekosystému plasty a jeho následné negativní dopady jsou ve srovnání s vodním prostředím stále spíše opomíjeny (Chae a An 2018; Schell et al. 2020). Nicméně, některé získané znalosti již teď umožňují posoudit význam používání plastů v zemědělství z hlediska půdní biogeochemie, agronomie a společnosti, a vedou k postupnému monitoringu půdy i z pohledu plastového znečištění (Scalenghe 2018).

2.6.1 Vliv na fyzikální vlastnosti

Kontaminace půdy plasty a plastovými fragmenty (MP i MBP) zhoršuje půdní vlastnosti čímž narušuje rovnováhu celého půdního ekosystému. Zhoršení vlastností zahrnuje porušenou půdní strukturu, snížení schopnosti tvořit agregáty, změnu pórovitosti půdy a zvýšení koncentrace znečišťujících látek v půdním roztoku (Rillig 2012; Huerta Lwanga et al. 2017b). Vliv plastů na vlastnosti půdy se liší v závislosti na typu a velikosti plastů. Například k degradaci půdní struktury obecně dochází v důsledku kontaminace makroplasty (Qi et al. 2020), což dále snižuje infiltraci a negativně tak ovlivňuje vodní kapacitu půdy a objemovou hmotnost půd (de Souza Machado et al. 2018b; Kim a An 2019). To může nakonec vést až anoxickým podmínkám, které jsou problematické například pro kořenový systém rostlin (Liu et al. 2014). MP interagují s různými složkami půdy a dostávají se do půdních agregátů; volné agregáty vznikají po interakci se surovými (odpadními) plasty a hrubé/kompaktní agregáty vznikají díky vláknitým MP (Rillig et al. 2017; Wang et al. 2020b). Používání plastových mulčovacích fólií v zemědělství způsobuje zhoršování stability půdních agregátů a brání provzdušňování půdy a propustnosti vody, což v konečném důsledku ovlivňuje růst kořenů a omezuje produkci plodin (Zhang a Liu 2018; Jiang et al. 2019). Studie autorů Wan et al. (2019) také uvádí, že plastové fólie o tloušťce 2, 5 a 10 mm vytvářejí mikrokanálky, které zvyšují rychlost evapotranspirace, a tudíž usnadňují vysychání půdy s následnými negativními účinky na růst rostlin (Wan et al. 2019). Jedním z mechanismů, který usnadňuje rychlejší výpar vody je patrně změna konformace supramolekulární struktury půdní organické hmoty (Fojt et al. 2022).

Vliv plastů na fyzikální vlastnosti půdy závisí také na typu plastů. Studie de Souza Machado et al. (2018) uvádí rozdílné účinky přídavku čtyř typů MP PP vlákna, PET vlákna, PE fragmenty a PA mikrokuličky) na vodní kapacitu, objemovou hmotnost a vodostálé agregáty (de Souza Machado et al. 2018b). Například objemová hmotnost a vodostabilní agregáty se výrazně snížily u půd ošetřených PET vlákny, zatímco ostatní typy plastů podobné výsledky neposkytly. Další studie autorů Boots et al. (2019) uvádí, že přídavek HDPE změnil stabilitu agregátů ve srovnání s biologicky rozložitelnou PLA a oděvními vlákny (Boots et al. 2019). de Souza Machado et al. (2019) v jiné studii uvádí, že PA a PES zvýšily rychlost odpařování vody z půdy více než HDPE, PET a PS (de Souza Machado et al. 2019). Zhang et al. (2019) na druhou stranu uvedli, že PET mikrovlákna neměla žádný vliv na objemovou hmotnost půdy, ale snížila její vodní kapacitu (Zhang et al. 2019a). Takovéto protichůdné účinky plastů mohou souviset s koncentrací kontaminujících MP a vlastnostmi půdy (Kim et al. 2021) protože například srovnávací studie vlivu podobně smáčivých polyesterových MP, jako je biodegradabilní poly-3-hydroxybutyrát (P3HB) a perzistentní PET, na vysychání vlhkosti z půdní organické hmoty (SOM), odhalila jen malé rozdíly v jejich nepříznivých účincích (Fojt et al. 2022).

Souhrnně tyto studie naznačují, že přídavek plastů do půdy ovlivňuje kontinuum půda-voda-atmosféra, což má v konečném důsledku dopad na vývoj plodin (Wan et al. 2019; Zhang et al. 2019a). Z toho vyplývá, že akumulace plastů v půdě může ovlivnit koloběh živin a hydrologické cykly, což v konečném důsledku může ohrozit potravinovou bezpečnost v souvislosti s globální změnou klimatu. Základní mechanismy, jejichž prostřednictvím plasty ovlivňují správné fungování půdy, jsou však stále nedostatečně prozkoumány a týkají se změny způsobu agregace půdních částic nebo re-konformace struktury SOM. Vliv přídavku plastů na tyto půdní vlastnosti proto poskytuje základ pro další pochopení jejich rizik na procesy v půdních ekosystémech.

2.6.2 Vliv na chemické vlastnosti

Plasty mají vliv nejen na fyzikální vlastnosti půdy, ale ovlivňují i její chemické vlastnosti, což v konečném důsledku ovlivňuje erodabilitu, úrodnost půdy a produkci plodin (Liu et al. 2017; Rillig et al. 2019a; Chen et al. 2020b). Nejvíce ovlivněnými chemickými vlastnostmi půdy v reakci na plasty jsou pH půdy, obsah SOM, transport organických a anorganických látek (často polutantů) a obsah živin v půdě. Půdní pH je jedním z hlavních faktorů určujících půdní vlastnosti včetně interakcí SOM a minerálů, dostupnosti živin pro rostliny a správného fungování mikrobiálních společenstev (Rousk et al. 2009). Přídavek plastů mění pH půdy, přičemž rychlost změny dále závisí na edafických faktorech a způsobech hospodaření.

Například autoři studie Lozano et al. (2021) zaznamenali zvýšení pH půdy v reakci na přídavek MP, které souviselo s obsahem půdní vlhkosti, přičemž pH půdy se zvýšilo za sucha (nižší obsah vlhkosti) (Lozano a Rillig 2020). V tomto ohledu jsou hlavním problémem při hospodaření se zemědělskou půdou zbytky plastových fólií, které mají vysoký potenciál pH půdy měnit. Bylo totiž zjištěno, že při rozkladu plastů se do půdy uvolňují aditiva i molekulární fragmenty, které mění chemismu půdy, ovlivňují půdní edafon, a tím i původní pH (Wang et al. 2020b). Příčinou změny pH je i velký povrch MP, který může měnit rychlost a velikost kationtové výměnné reakce, což dále umožňuje volnou výměnu kationtů s půdní vodou (Boots et al. 2019). Kromě toho se ukazuje, že MP ovlivňují proces nitrifikace uvolňováním iontů H^+, což vede je značnému okyselení půdního prostředí (He et al. 2021; Wang et al. 2022a). Nicméně, několik autorů pozorovalo zvýšené pH kontaminované půdy. Například autoři studie (Yang et al. 2021) zaznamenali zvýšení pH po přídavku 1 % PLA a 10 % vysokohustotního HDPE (Yang et al. 2022a).

Změny pH mohou mít negativní vliv na biodiverzitu půdního mikrobiomu, schopnost půdy poskytovat živiny rostlinám, ale i například imobilizaci Al^{3+}, který je fytotoxický. Obecně lze vypozorovat, že pro pochopení účinků plastů na pH půdy je nutné zohlednit především původní vlastnosti půdy, typ plastů, koncentraci ale i dobu expozice.

Dalším z problémů spojených s používáním plastů v půdě je degradace půdní organické hmoty (SOM) (Hodson et al. 2017). MP mohou mít různé účinky na SOM, od snížení až po podporu akumulace SOM prostřednictvím změněné mikrobiální aktivity a dostupnosti živin (Cao et al. 2017; Liu et al. 2017; Chen et al. 2020a; Kim et al. 2021). Většina studií se soustředila na rozpuštěnou organickou hmotu (DOM), která je klíčovou součástí SOM důležitou pro koloběh C v půdě, transport znečišťujících látek a dynamiku živin (N a P) (Kalbitz et al. 2000; Feng et al. 2021). Přídavek MP často snižuje množství DOM (Yu et al. 2022), dynamika DOM při přídavku MP závisí na čisté bilanci mezi produkčním a mineralizačním potenciálem SOM (Liu et al. 2017). Jiné výzkumy naproti tomu uvádí, že vysoké koncentrace (28 % hm.) PP-MP výrazně zvyšují obsah DOM a dalších živin (N a P) v půdě ve srovnání s nižšími koncentracemi (14 % hm.), což je dáváno do souvislsoti s vlivem PP na činnost půdních enzymů zapojených do koloběhu C, N a P (Fei et al. (2020)) prokázali významný vliv MP na aktivity enzymů koloběhu živin.

Tato zjištění podporují snahy o vypracování kritických mezí pro kvantifikaci účinků MP na ukazatele kvality půdy (Fei et al. 2020). Tato zjištění také naznačují potřebu komplexnějších studií zohledňujících změny v dostupnosti a/nebo fixaci živin aplikovaných na povrch půdy v reálném čase. Dosavadní studie navíc uváděly pouze účinky MP na hlavní půdní živiny, v budoucnu by mělo být provedeno více studií zohledňujících účinky MP na mikroživiny.

2.6.3 Vliv na biologické půdní vlastnosti

Kromě vlivu na fyzikálně-chemické vlastnosti půdy ovlivňují MP také biologické vlastnosti půdy, což má v konečném důsledku vliv na ekosystémové služby půdy. V toto ohledu je půdní mikrobiální aktivita považována za jednu z nejdůležitějších procesů (Jung et al. 2010; Bender et al. 2016) protože transformace a koloběh půdních živin (C, N, P a S) vyžaduje správnou rovnováhu mezi strukturou a fungováním půdních mikrobiálních společenstev a jejich extracelulárních enzymů. MP mohou ovlivnit mikrobiální metabolismus v půdě, což v konečném důsledku narušuje její zdraví a další biologické procesy (Burns a Boxall 2018; de Souza Machado et al. 2018a).

Mikrobiální extracelulární enzymy jsou prvotními indikátory úrodnosti půdy, protože jejich aktivita zajišťuje mikrobům dostupnost živin (Liu et al. 2017), nicméně neexistuje obecná shoda ohledně koncentrace nebo úrovně kontaminace, při které MP způsobují negativní nebo pozitivní účinky na aktivitu půdních enzymů. Například studie autorů (Huang et al. 2019) a (Yang et al. 2018) uvádějí, že přídavek MP může významně zvýšit aktivitu katalázy, ureázy, fenoloxidázy a půdní fosfatázy. Naproti tomu, autoři studie (Fei et al. 2020) uvádějí, že MP PE a PVC-MP vykazují inhibiční účinky na aktivitu fluorescein diacetát hydrolázy (FDAse), která je důležitým ukazatelem krátkodobých změn kvality půdy (Muscolo et al. 2015) a naopak, studované MP podporovaly aktivitu kyselé fosfatázy a aktivitu ureázy. Kromě toho bylo prokázáno (de Souza Machado et al. 2018), že PP a polyesterová mikrovlákna mohou mít inhibiční účinky na hydrolytickou aktivitu fluorescein diacetátu (de Souza Machado et al. 2018a). To naznačuje, že nejen koncentrace/úroveň kontaminace, ale také typ plastů může ovlivňovat půdní enzymy. Proto je nutný další výzkum zaměřený na stanovení prahových hodnot, u kterých lze hodnotit pozitivní a negativní účinky plastů.

Plasty mohou ovlivňovat půdní mikrobiom včetně bakterií a hub (Wijesekara et al. 2018). Dřívější studie zkoumaly vliv MP na bakteriální znaky, včetně transportu bakterií (MP jsou vektorem), šíření genů rezistentních vůči antibiotikům a mikrobiálního metabolismu (Horton et al. 2017; Arias-Andres et al. 2018; Bradney et al. 2020). MP mohou mít schopnost přenášet mikrobiální invazní druhy (Trojan et al. 2024), nicméně úloha znečištění MP při transportu mikrobů není dostatečně prozkoumána. Pouze několik studií se zaměřilo na transport mikrobů a genetickou výměnu, například He et al. (2018) uvedli zvýšený transport bakterií (*E. coli*) částicemi PS v křemenném písku při zvyšující se iontové síle. Bylo také zjištěno, že MP poskytují povrch pro výměnu genů a dalších metabolických produktů mezi fylogeneticky nepříbuznými mikroorganismy, jak již dříve ukázali (Sun et al. 2018; Huang et al. 2019). Kromě neutrálních genů jsou MP odpovědné za šíření škodlivých genů, jako jsou ARG (antibiotic resistance genes, geny antibiotické rezistence), ohrožujících lidské zdraví (Arias-Andres et al. 2018; Imran et al. 2019). Tyto indicie dokazují, že kontaminace půdy MP může ovlivnit mikrobiální metabolismus, mikrobiální enzymy a jejich fungování, což potenciálně ovlivňuje zdraví půdy.

Plasty, především MP, představují kolonizovatelný povrch pro mikroorganismy a podle svých fyzikálně-chemických vlastností usnadňují mikrobiální adhezi (Ghosh et al. 2019; Miao et al. 2019). Takováto asociace MP s mikroby může buď zvýšit jejich škodlivý vliv na živé organismy (Lu et al. 2019; Wang et al. 2021) nebo umožnit a urychlit jejich rozklad (Orr et al. 2004; Nevius et al. 2012) a rozptýlení v životním prostředí. Spojení MP s mikroby v životním prostředí ovlivňují různé fyzikální, chemické nebo biologické faktory. Bylo zjištěno, že na půdní MP se vážou různé mikroorganismy jakými jsou houby (Sabev et al. 2006; Cosgrove et al. 2007; Rüthi et al. 2020; Zhang et al. 2020), protisty (Sabev et al. 2006; Oksińska et al. 2019) a nejčastěji pak bakterie (Bailes et al. 2013; Zhang et al. 2019b; Kavitha a Bhuvaneswari 2021; MacLean et al. 2021). Pro mikroorganismy je adheze na MP výhodná, protože zvyšuje přístup k živinám akumulovaným na povrchu MP v důsledku zvýšené adherence (Tuson a Weibel 2013), což pak dále stimuluje mikrobiální růst (Free et al. 2014; Shen et al. 2019).

Mikrobi, zejména bakterie, se na plastové povrchy často přichycují prostřednictvím tvorby biofilmu (Free et al. 2014; Shen et al. 2019). Tvorba biofilmu je obecně zprostředkována extracelulárními polymerními látkami (EPS) (Douterelo et al. 2014; Ramsperger et al. 2020). Tvorba biofilmů na MP ve vodním prostředí byla hojně uváděna různými autory (Douterelo et al. 2014; Miao et al. 2019; Wu et al. 2019; Ramsperger et al. 2020; Rosato et al. 2020), pro půdu jsou však důkazy prozatím poměrně vzácné (Mercier et al. 2017; Han et al. 2020; Sarker et al. 2020; Kavitha a Bhuvaneswari 2021). Tvorba biofilmu může vyvolat změny ve struktuře a funkcích mikrobiálních společenstev jak v rámci stávajících mikrobiálních společenstev, tak mezi nimi (Miao et al. 2019; Yuan et al. 2020). Jedinečná mikrobiální společenstva navázaná na MP byla označena jako "plastisféra" (Zettler et al. 2013). Charakteristiky plastisféry se mohou měnit s velikostí plastů, kdy podle Debroas et al. (2017) alfa- a gama-proteobakterie dominují na mezoplastech PET a PS (5 mm až 20 cm) ve srovnání s MP (300 µm až 5 mm), zatímco (Zhang et al. 2017) pozorovali obohacení Actinobacteria na MP (0,1–5 mm) PP, PE a PS (ve srovnání s mezoplasty, 5–25 mm).

Dále bylo zjištěno, že biofilmy způsobují strukturální a funkční změny MP způsobené uvolňováním enzymů degradujících/modifikujících MP, maskováním povrchových vlastností, vypouštěním vedlejších metabolických produktů a degradací aditiv a to včetně PTE (potentially toxic elements) hlavně kovů a perzistentních organických polutantů adsorbovaných na MP

(Miao et al. 2019). Díky těsnému kontaktu mezi povrchem bakterií a (mikro)plastů jsou MP/NP schopny pronikat plazmatickou membránou a hromadit se v živých mikrobiálních buňkách. Protože MP/NP jsou většinou ve vodě nerozpustné syntetické polymery, jsou náchylné k fragmentaci a přičemž při dostatečně malém rozměru mohou být mikroorganismy pohlceny (Beiras et al. 2018). Mezi způsoby interakce/akumulace MP/NP v mikroorganismech patří bioadsorpce na buněčný obal, buněčné vychytávání a biodegradace. Mechanismus buněčného příjmu a akumulace MP/NP je ovlivněn jak fyzikálně-chemickými vlastnostmi syntetických materiálů (např. jejich velikostí, tvarem, hydrofobností a polaritou), tak vlastnostmi mikrobiální buněčné membrány (Mammo et al. 2020).

Kromě přímých interakcí mezi částicemi MP a půdními mikroorganismy vznikají obavy o životní prostředí kvůli chemickým látkám, které jsou v MP obsaženy (např. antioxidanty, stabilizátory) a také kvůli znečišťujícím látkám, které se do MP dostávají z kontaminovaného prostředí. Bylo například zjištěno, že biofilmy na povrchu MP slouží jako přenašeče různých znečišťujících látek (Wang et al. 2021). MP mohou působit jako nosiče různých hydrofobních organických chemických látek (HOC, např. 1,2,4,5-tetrachlor-o-benzenu – TeCB, pentachlorbenzenu – PeCB nebo hexachlorbenzenu – HCB), usnadňují přenos HOC do bioty v různých koncentracích v závislosti na tom, jaké jsou podmínky prostředí, které řídí (de)sorpci (Hartmann et al. 2017). Plastisférická mikrobiální společenstva mohou také selektivně zvýhodňovat patogeny a přispívat k jejich přežívání a šíření v prostředí. V biofilmech asociovaných s MP ve vodě se vyskytují *V. parahaemolyticus* (Kirstein et al. 2018) a *V. cholerae* (Silva et al. 2019) a další pro člověka potenciálně patogenní mikroby (*Aeromonas, Haemophilus, Acinetobacter* (Virsek et al. 2017), *Pseudomonas monteilii, P. mendocina* (Wu et al. 2019), patogenní *Escherichia coli* (Silva et al. 2019), přičemž byl prokázán i rostlinný patogen *Pseudomonas syringae* (Wu et al. 2019). (Mikro)plastické biofilmy navíc díky zvýšené dostupnosti živin a vyšší hustotě buněk představují "hotspots" pro horizontální přenos genů (HGT) (Aminov 2011; Arias-Andres et al. 2018). K HGT dochází především prostřednictvím konjugace (Aminov 2011; Arias-Andres et al. 2018). Byl prokázán přenos plazmidů z *Pseudomonas sp.* na *E. coli* a z *E. coli* na *Arthrobacter* na biofilmech asociovaných s MP (Arias-Andres et al. 2018).

Z těchto důvodů může zvýšená HGT v biofilmech usnadňovat šíření ARG v přijímajícím prostředí (Suzuki et al. 2017). Dlouhodobé vystavení bakterií kontaminantům adsorbovaným na MP, jako jsou antibiotika a kovy, může přispívat ke změnám jejich profilu rezistence vůči antibiotikům (Huijbers et al. 2015; Singer et al. 2016; Laganà et al. 2019). V důsledku toho byly ARG nalezeny častěji na plastech ve srovnání s okolním prostředím, což naznačuje, že plasty mohou hrát zásadní roli ve vývoji a šíření rezistence vůči antibiotikům. Autoři práce (Yang et al. 2019) prokázali 13 různých genů ATB rezistence (ARG) v mikrobech adsorbovaných na plastech a k antibiotikům rezistentní izoláty *Vibrio spp.* (Laverty et al. 2016) a druhů *Pseudomonas* (Wu et al. 2019; Laverty et al. 2016).

MP, díky své převážně lipofilní povaze, mohou na svém povrchu adsorbovat různé nepolární polutanty ze skupiny léčiv, polychlorovaných bifenylů (PCB), perzistentních organických polutantů (POP), polycyklických aromatických uhlovodíků (PAH) a těžkých kovů (Ni, Ti, Pb, Zn, Cd, Cu) (O'Donovan et al. 2018; José a Jordao 2020). Ve spolupráci s mikroorganismy tvořícími biofilm působí MP jako zdroj a vektor škodlivých polutantů, jako jsou perzistentní organické polutanty (Pittura et al. 2018) a těžké kovy (Brennecke et al. 2016), a biofilm mění adsorpční chování MP (Wang et al. 2020c).

Kromě mikroorganismů mohou být přítomností MP/NP v půdě ovlivněny i vyšší půdní organismy. V porovnání s vodními organismy, u nichž bylo v posledních letech získáno více poznatků o možných negativních účincích MP/NP ve vodě, jsou však environmentální důsledky pro půdní mezo- a makrofaunu vystavenou působení MP/NP méně prozkoumány. Obecně je o koncentracích MP/NP v půdě a skutečných úrovních expozice známo poměrně málo, ale bylo zjištěno, že přítomnost mikročástic plastů může narušovat pohyb půdních organismů, jako jsou např. chvostoskoci (Kim a An 2019, Maass et al. 2017), zatímco některé půdní organismy, jako jsou žížaly (Huerta Lwanga et al. 2017a; Rillig et al. 2017), mohou ovlivňovat transport MP/NP v půdě a přesouvat je vertikálně, což znemožňuje foto- a termickou degradaci, které jsou klíčové pro zmenšení velikosti polymerů před biologickým rozkladem. Kromě toho se v hlubších vrstvách půdy mohou vytvořit anaerobní podmínky a potlačit oxidační degradační procesy (Thomas et al. 2012). Kromě toho, jak ukázali (Huerta Lwanga et al. 2016), bakterie ve střevě *Lumbricus terrestris* mohou rozkládat MP, zdá se pravděpodobné, že částice přijaté na povrchu jsou v hlubších vrstvách vylučovány jako menší částice. Půdní mezo/makroorganismy tedy mohou alespoň do určité míry ovlivňovat osud MP/NP v půdě a stejně tak mohou MP/NP představovat účinky na půdní organismy. Například organismy, jako jsou žížaly, by mohly MP/NP ve velké míře pozřít, což může způsobit poškození jejich střevního traktu a snížit míru jejich přežití (Fahrenkamp-Uppenbrink 2016).

MP/NP mohou také způsobovat neurotoxicitu (Qu et al. 2019b), oxidační poškození a další nepříznivé účinky (např. poškození střev, (Qu et al. 2019a), reprodukční toxicitu u organismů, jako je *Caenorhabditis elegans*, kde bylo prokázáno, že čím menší je velikost plastových částic, tím vyšší je expozice bioty (Lei et al. 2018). MP/NP mohou ovlivňovat společenstva hlístic, kde byl pozorován negativní dopad (z hlediska početnosti) v pořadí: houbami/bakteriemi se živící hlístice (bez významného dopadu) < hlístice živící se rostlinami ~ dravé hlístice < všežravé hlístice (Lin et al. 2020). Byly pozorovány transgenerační účinky v důsledku expozice nano PS částicím (Zhao et al. 2017), pravděpodobně v důsledku translokace nano PS částic do reprodukčních orgánů, jako je gonáda, což potenciálně následně vedlo k přenosu nano PS částic na další generaci.

Pro studium účinků MP byly nejčastěji vybrány PE a PS mikrosféry, které představují snadno dostupnou kombinaci typu a tvaru plastu. Ty ale tvarem nedorážejí realitu vstupu plastů do půdních ekosystémů, protože podle (Zhang a Liu 2018) jsou převládající formou plastů v půdách vlákna, která tvoří v průměru 92 %, následovaná fragmenty a fóliemi, které přispívají 8 %. Vlákna MP v půdním prostředí jsou však ale prakticky neprozkoumány. Kromě toho by se budoucí studie měly přednostně zaměřit na další MP kromě PET a PS, protože podle (Xu et al. 2020) tvořily většinu polymerů PA a PP s menším podílem polyvinylchloridu (PVC) a PET v půdách. Na druhou stranu, při studiu účinků různých plastů včetně biologicky rozložitelných PLA a polypropylenkarbonát (PPC) a nerozložitelných (PE) dospěli autoři práce (Ding et al. 2021) k závěru, že při regulaci reakcí žížal na kontaminaci půdy je důležitá spíše koncentrace MP než typ plastu. Existují také další vlastnosti MP/NP, jako je stárnutí, povrchová úprava a přísady, které dosud nebyly dostatečně zdokumentovány (Büks et al. 2020). Nicméně i přes tato omezení se opakuje vzorec aktivního příjmu, po němž následuje změna populace v rámci střevního mikrobiomu a nepříznivé účinky na růst, metabolismus, reprodukci a mortalitu v různých kombinacích, zejména při vysokých koncentracích a malých velikostech částic.

2.6.4 Vliv na růst rostlin

Jak již bylo uvedeno výše, využití plastových výrobků v zemědělství se pozitivně projevuje na výnosu rostlin, nicméně po rozpadu nebo při jejich zanechání svému osudu kontaminují tyto plasty půdu, přičemž rozsah a závažnost nepříznivých účinků na rostliny vzrůstá s klesající velikostí plastového fragmentu (Qi et al. 2018; Wirnkor Verla et al. 2020).

MP v zemědělství ovlivňují růst a výkonnost rostlin buď nepřímo nebo přímo (Khalid et al. 2020; Leifheit et al. 2021; Yu et al. 2021):

i) nepřímo prostřednictvím narušení struktury půdy (praskání, změny pórovitosti, agregace, objemové hustoty) (Niu et al. 2016; Wan et al. 2019) a prostřednictvím změny dalších fyzikálně-chemických vlastností (pH, výpar, vodní kapacita, provzdušnění, elektrická vodivost (Niu et al. 2016; Lozano a Rillig 2020; Amare a Desta 2021)) nebo prostřednictvím poškození organismů žijících v půdě (Leifheit et al. 2021),

ii) přímo prostřednictvím ucpávání pórů semen (Bosker et al. 2019), prostřednictvím příjmu přes kořeny (nanočástic plastů o velikosti <0,1 μm) (Teuten et al. 2009; Azeem et al. 2021), transportu do výhonků (listů a stonků) (Su et al. 2019; Li et al. 2020), průchodu buněčnými membránami a inhibice nadzemního i podzemního růstu ve vegetativním i reprodukčním stádiu (Qi et al. 2018).

Nicméně, nepřímé účinky na rostliny mohou být jak pozitivní, tak negativní: MP (např. mikrovlákna) snižují objemovou hmotnost půdy čímž zvyšují její provzdušnění (de Souza Machado et al. 2018b), snižují odpor proti prorůstání kořenů porporujíce růst rostlin (Rillig et al. 2019b). Mikrovlákna jsou nejúčinnější v procesu tvorby agregátů: podle Zhanga a Liu (2018) se až 72 % MP v půdě podílí na tvorbě agregátů (Zhang a Liu 2018). Snížení objemové hmotnosti vlivem mikrovláken však může být selektivně prospěšné pro některé rostliny a ale škodlivé pro jiné (roste zranitelnost vůči stresu suchem), což vede k inhibici alelopatie a změnám ve společenstvu a diverzitě autochtonních lučních rostlin (Lozano a Rillig 2020). Změny hustoty a struktury půdy, připisované kontaminaci půdy MP, mohou souviset se změněnou aktivitou půdní bioty, např. změny hustoty stěn nor žížal (Huerta Lwanga et al. 2017a) nebo zlepšení zásobování rostlin vodou houbami arbuskulární mykorhizy (AMF) (díky menším půdním pórům) (Entry et al. 2002). Dále pak, bylo ukázáno, že zvýšená mortalita žížal v důsledku PE prášku nepřímo snižovala obsah vody a tím potenciálně potlačovala růst rostlin (Huerta Lwanga et al. 2017b). MP mění řadu půdních vlastností, které určují životní prostor pro AMF, a následně může dojít ke změně funkcí zprostředkovaných AMF (půdní agregace, transport vody a živin), které zmírňují růst a ovlivňují zdraví rostlin (Leifheit et al. 2021). Dále, mikrovlákna umožňují vytvoření vodních kanálků, které vedou jak k rychlému pronikání vody do hlubších vrstev půdy, tak ke zvýšené suchosti na povrchu půdy (Wan et al. 2019). Navíc, autoři práce Boots et al. (2019) zjistili, že kontaminace LDPE snižuje růst kořenů *Lolium perenne* (Boots et al. 2019). Kontaminace půdy plasty tedy významně ovlivňuje obsah vody v půdě a následně vodní režim rostlin (Yu et al. 2021) a významně brzdí růst rostlin a účinnost odstraňování N v mokřadu (Yang et al. 2020).

Dalšími vlastnostmi, které se v půdách kontaminovaných MP (PLA, LDPE) lišily, byly např. pH a elektrická vodivost EC (Qi et al. 2020; Wang et al. 2020b). Kontaminace půdy HDPE (vysokohustotním PE) také snížila pH půdy, což mohlo mít vliv na mikrobiální růst (Bandow et al. 2017; Boots et al. 2019), složení a diverzitu půdního mikrobiálního společenstva (Rousk et al. 2009). Zejména degradace biodegradabilních polymerů a tvorba degradačních produktů (např. kyseliny 3-hydroxymáselné z PHBV) může snížit pH a změnit půdní podmínky ve prospěch specifických taxonomických skupin, např. acidobakterií (Zhou et al. 2021a), a tím může modifikovat podmínky pro růst rostlin. Kromě toho zvýšení mikrobiální aktivity způsobené biodegradací P3HB způsobuje konkurenci mezi rostlinami a mikroorganismy o živiny, což vede k potlačení růstu rostlin (Brtnicky et al. 2022). Mohlo by se tedy jednat o domnělou změnu růstových podmínek v půdě kontaminované MP, nicméně pouze ojedinělý výzkum (Wang et al. 2020a) poukazoval na významnou fytotoxicitu zprostředkovanou MP (např. vysokou dávkou PLA nebo jiných degradačních produktů).

Dále se spekuluje, že snížená mikrobiální aktivita po kontaminaci MP (nebo NP po dalším rozpadu) by mohla mít přímý dopad na růst rostlin a plodin (Powell a Rillig 2018), co může být ovlivněno menší početnost a změnou struktury společenstva AMF (Wang et al. 2020a). MP z PLA ovlivňují některé z mikrobiálních druhů (např. mikrobiální oxidanti amoniaku) v půdě, což může ovlivnit ukládání půdního organického C a N (Chen et al. 2020a) a také míru asistované mikrobiální absorpce minerálních látek a fixace N (Fei et al. 2020). Naopak MP PP nepříznivě ovlivňují půdní enzymy, jako je ureáza, glukosidáza a fosfatáza, zatímco vliv na degradaci glyfosátu nebyl prokázán (Yang et al. 2018).

K přímému působení MP (nebo NP) na rostliny dochází v důsledku vstupu a akumulace MP (o velikosti ≤100 nm) do těla a buněk rostliny. MP/NP ovlivnily, většinou negativně, klíčení semen a to ucpáním pórů v semenech (Bosker et al. 2019), snížením životaschopnosti semen (Li et al. 2021a), sníženým příjmem vody (Yuan et al. 2019), nižší klíčivostí (Boots et al. 2019), snížením délky kořenů během klíčení (Giorgetti et al. 2020). Na druhou strany, byl ale také pozorován i neutrální účinek s následným zvýšeným růstem semenáčků pšenice (PS NP) (Lian et al. 2020). Ovlivněn byl i růst, výška a biomasa kořene, jak bylo uvedeno u *Lepidium sativum* (v důsledku akumulace na kořenových vláskách) (Bosker et al. 2019), podobně byly MP extracelulárně zachyceny ve slizu kořenové čepičky u salátu (Li et al. 2019) nebo se zpočátku akumulovaly v kořenech před transportem do nadzemních částí rostlin okurky (Li et al. 2021b). Nepříznivý vliv MP na kořeny byl dále zaznamenán u rostlin rýže (Dong et al. 2020), fazole mungo (Chae a An 2018), cibule (de Souza Machado et al. 2018a), orobince širolistého (*Typha latifolia*) vysazeného v mokřadech (Yang et al. 2020), bobu (*Vicia faba*) (Jiang et al. 2019), pšenice cestou crack-entry (Li et al. 2020), vodní rostliny *Lemna minor* (Kalcikova et al. 2017). Akumulace byla také závislá na povrchovém náboji MP, kladně nabité NP se hromadily v kořenových špičkách, záporně nabité NP byly pozorovány v apoplastu a xylému *Arabidopsis thaliana* (Sun et al. 2020).

Ve většině případů bylo demonstrováno, že nejprve došlo k akumulaci NP v kořenech a následně k jejich distribuci do celého těla rostliny, přičemž nejsilnější dopad měly na růst výhonků a biomasu (Jiang et al. 2019; Li et al. 2020; Lian et al. 2020). Nicméně, byl zjištěn i opačně směrovaný tok MP s listovým příjmem a translokací z listů do kořenů u kukuřice (Sun et al. 2021). Na genotoxické a oxidační poškození, způsobené v rostlinných tkáních NP, reagovaly rostliny změnou obsahu chlorofylu v listech (Kalcikova et al. 2017; Dovidat et al. 2019; Gao et al. 2019b; Mateos-Cardenas et al. 2019; Sun et al. 2020; Wang et al. 2020b) a aktivitou antioxidačních enzymů (Jiang et al. 2019; Sun et al. 2020; Yang et al. 2020). NP PS změnila hladiny Mg, Ca, Fe v plodech okurek a zvýšila obsah prolinu v kořenech v rostlinách okurek (Li et al. 2021b). Dalšími ukazateli oxidačního stresu vyvolaného MP v porostu a kořenech rostlin (rýže *Oryza sativa* L.) byly zvýšené hladiny volných radikálů (reactive oxygen species, ROS), zvýšení bylo zaznamenáno po ošetření PE mulčovací fólií oproti biologicky rozložitelné fólií na bázi PBAT (Yang a Gao 2022). Stresový způsobený MP může také snižovat expresi transportérů amoniaku a dusičnanů u rýže (AMT3.3, NRT2.1, NRT2.1) (Yang a Gao 2022). Menší pozornost byla zatím věnována stresovým účinkům biodegradabilních plastů, ačkoli existují studie naznačující, že 0,1 % PLA MP významně snižuje délku kořenů sóji (*Glycine max* Merr.), což bylo spojeno jak se snížením aktivity antioxidačních enzymů, tak se zmírněním metabolické dráhy aminokyselin je metabolismus (Lian et al. 2022).

2.6.5 P3HB

Tato disertační práce studuje vlivy biodegradabilních plastů na půdu a růst rostlin. Jako modelový zástupce byl vybrán P3HB, zástupce ze skupiny polyalkanoátů, jehož vlastnosti jsou zde uvedeny pouze ve zkratce, důkladnější rešerše je potom prezentována v již publikovaných pracích.

Polyhydroxyalkanoáty (PHA) představují skupinu přírodních biodegradabilních plastů (BP), které jsou považovány za perspektivní materiály jedna díky svým fyzikálně-chemickým a mechanickým vlastnostem, možnosti termoplastického zpracování (Alcântara et al. 2020) a také schopnosti podléhat jak aerobní, tak anaerobní biodegradaci (Sehgal a Gupta, 2020; Shah a Kumar, 2021). Mezi nejrozšířenější a nejvíce studované zástupce této skupiny patří polyhydroxybutyráty (PHB), zejména P3HB a jeho kopolymer poly(3-hydroxybutyrát-co-3-hydroxyvalerát) (PHBV) (Fuessl et al. 2012). PHB lze nicméně syntetizovat i z jiných monomerů například 2-HB a 4-HB což umožňuje vznik polymerů různých délek monomerních řetězců, včetně poly-2-HB, poly-3-HB a poly-4-HB (Sudesh et al., 2000).

P3HB, jako nejčastěji studovaný PHA, je syntetizován celou řadou prokaryotických bakterií, včetně půdních, za podmínek nerovnováhy živin (přebytek C a nedostatek N)

(Grousseau et al. 2013; Lee 1996) jako zásobní molekula uhlíku a zdroj energie (Alves et al., 2017), což jej činí velmi vhodným pro široké spektrum aplikací (Yu et al., 2006; Albuquerque et al., 2020).

Biodegradace P3HB probíhá v mikrobiálně aktivním prostředí, včetně sladkovodních a mořských ekosystémů (Briassoulis et al., 2019), anaerobních kalů (Cazaudehore et al., 2023), sedimentů (Eich et al., 2021) a půdě (Serrano-Ruiz et al., 2023). Rychlá a úplná rozložitelnost je klíčovou vlastností, díky níž jsou PHA, zejména P3HB, považovány za environmentálně příznivé polymery (Vroman a Tighzert, 2009; Luckachan a Pillai, 2011) a vhodnou alternativu ke konvenčním, nerozložitelným plastům (Bonartseva et al., 2003). Enzymy podílející se na biodegradaci P3HB, jako jsou depolymerázy a hydrolázy, byly identifikovány u několika mikrobiálních taxonů (Kadouri et al., 2003; Shah et al., 2007; Panayotidou et al., 2014; Roohi a Kuddus, 2018). PHB-depolymerázy jsou zodpovědné za štěpení polymerních řetězců na monomer kyseliny 3-hydroxymáselné, která je dále plně využitelná mikroorganismy (Altaee et al., 2016; Jendrossek et al., 2002; Kozlovskii et al., 1999).

P3HB v posledních letech stále více zkoumán jako polymer využitelný v různých zemědělských aplikacích. Rostoucí zájem o udržitelné zemědělské technologie podporuje využívání P3HB jako zatím sice ekonomicky nevýhodného, ale potenciálně ekologicky příznivého materiálu (Ngo, 2020). Nicméně s rostoucím uplatněním PHA v zemědělských aplikacích může narůst jejich koncentrace v půdním prostředí, přičemž v případě využití jako mulčovací fólie, by mohly zbytkové koncentrace překročit 1,5 % (Palucha et al., 2024). To samozřejmě vyvolává obavy ohledně jejich potenciálního dopadu na agroekosystémy.

Konkrétní známé aplikace P3HB v zemědělství zahrnují mulčovací materiály (Kaisrajan a Ngouajio, 2012), coating hnojiv pro řízené uvolňování (Volova et al., 2016), nosičové systémy pro mikroorganismy (Boyandin et al., 2016), a také použití v květináčích a výsevních páscích či při aplikaci agrochemikálií s řízeným uvolňováním (Touchaleaume et al., 2016; Vroman a Tighzert, 2009).

2.6.6 Vliv P3HB na půdní vlastnosti

P3HB se z a příznivých podmínek biologicky rozkládá poměrně rychle, a to v řádu týdnů až měsíců (Kawashima et al., 2019). Nicméně, příznivé podmínky se vyskytují především v půdách s vyšším obsahem půdní organické hmoty (SOM), dostatkem živin, vyšší mikrobiální aktivitou, a vhodným nasycením vodou. Přirozená koncentrace P3HB v půdě kolísá, přirozené množství je v mikrogramech na gram mikrobiálního uhlíku (Elhottova et al., 2000). V půdě je P3HB využíván širokou škálou mikroorganismů, včetně saprofytických hub (Altaee et al.,

2016) a bakterií (Volova et al., 2017), a proto tvoří přirozenou součást půdního potravního řetězce (White et al., 2021). Z tohoto důvodu, nadměrné množství P3HB částic může vyvolávat nežádoucí účinky a to především snižovat dostupnost organické hmoty pro mikroorganismy (Pathan et al., 2020) ovlivňovat distribuci živin (Zhao et al., 2021)včetně kyslíku (Brtnicky et al., 2024b) a tím narušovat půdní potravní řetězec (Rillig et al., 2019), stimulovat půdní respiraci a zvyšovat emise CO_2 (Liu et al., 2019), měnit složení mikrobiálního společenstva ve prospěch druhů schopných jeho degradace (Fernandes et al., 2020) a snižovat pH půdy (Volova et al., 1998). S ohledem na fyzikální vlastnosti půdy, MP z P3HB zvyšují rychlost odpařování vody a podporují vysychání půdy, podobně jako MP z PET (Fojt et al., 2022) a to změnou supramolekulární struktury SOM, což ovlivňuje vodní kapacitu (WHC), pohyb vody i její dostupnost pro rostliny. Vyšší koncentrace P3HB v půdě proto nejsou bez rizika, jak se dříve předpokládalo.

Patrně nejzásadnějším problémem je stechiometrická nerovnováha způsobená nadbytkem snadno dostupného uhlíku (Brown et al., 2023), vzhledem k tomu, že optimální poměr C:N v půdě je ca 7–8,6 (Manzoni et al., 2017; Spohn, 2015). Výsledný nárůst tohoto poměru vede ke zvýšené poptávce po N, který mikroorganismy získávají z SOM pomocí produkce N-hydroláz (Zhu et al., 2021). Tímto způsobem dochází k intenzivnějšímu využití zdrojů a ke zpřístupnění N, který by jinak byl dostupný rostlinám, s možnými negativními důsledky pro kvalitu SOM (Brown et al., 2023; Serrano-Ruiz et al., 2023). Mikrobiální společenstva na změnu poměru C:N také reagují šířením degradérů, kteří následně ovlivňují strategie rostlin i složení rhizobiomu. Přestože několik studií toto téma zkoumalo (Altaee et al., 2016; Serrano-Ruiz et al., 2023; Volova et al., 2022; Zhou et al., 2021a), stále není jasné, jak míra biodegradace, množství zbytků plastu a aktivita degradátorů souvisí s konkrétními půdními parametry a toky živin.

Schopnost půdy degradovat P3HB do značné míry závisí na jejím biotickém složení – jak na makroorganismech (např. žížaly, Sanchez-Hernandez et al., 2020), tak na mikrobiálních společenstvech (Abou-Zeid et al., 2004; Rychter et al., 2006). Právě struktura mikrobiálního společenstva bývá označována jako klíčový faktor rychlé a účinné biodegradace (Guo et al., 2010; Vogel et al., 2021). K degradačně aktivním mikroorganismům patří saprofytické houby (Sang et al., 2002; Altaee et al., 2016) a bakterie z rodů *Bacillus, Paenibacillus, Streptomyces, Arthrobacter, Azospirillum a Pseudomonas* (Ito et al., 1998; Manna et al., 1999; Volova et al., 2017), včetně některých rhizobakterií (Bonartseva et al., 2003; Kadouri et al., 2003; Jeszeova et al., 2018). Aktivita mikrobů degradujících P3HB je proto úzce vázána na rovnováhu a dostupnost živin v půdě. Rozklad probíhá efektivněji při dostatku limitujících prvků, především N (Nishide et al., 1999; Sang et al., 2004; Muneer et al., 2020; Zhou et al., 2021c).

Přítomnost PHA také ovlivňuje půdní mikroorganismy prostřednictvím změn v taxonomickém i funkčním složení společenstva (Brown et al., 2023; Dey a Tribedi, 2018; Sang et al., 2002; Zhou et al., 2021a). Dochází k obohacení půdy o PHA-degradující taxony, jako jsou *Alphaproteobacteria* (Lian et al., 2022; Liu et al., 2023), *Actinobacteria* (Lian et al., 2022; Liu et al., 2023; Meng et al., 2021), *Ascomycota* (Liu et al., 2023), pravděpodobně i *Gammaproteobacteria* (Chen et al., 2020). Tyto změny vedou k posunům v abundanci funkčních skupin, například kopiotrofů (Rüthi et al., 2020), oligotrofů (Moore-Kucera et al., 2014), nitrifikačních prokaryot (Di Mola et al., 2021) a *Firmicutes* (Ong a Sudesh, 2016). Mezi rozpoznané PHA-degradující houby patří zástupci fyla *Ascomycota* (Šerá et al., 2020; Matavulj a Molitoris, 1992; Tanunchai et al., 2021), *Basidiomycota* (Matavulj a Molitoris, 1992) a *Deuteromycetes* (Lee et al., 2005). Vyšší zastoupení skupiny *Nitrospirae* po přídavku PHBV poukazuje na změny v koloběhu N (Zhou et al., 2021).

Jen málo studií (Barak et al., 1991; Boyandin et al., 2011; Palucha et al., 2024) se detailně zabývá vztahem mezi mírou biodegradace P3HB a rozkladem autochtonní SOM. Například autoři studie Palucha et al. (2024) zaznamenali po několika měsíčním experiment pokles SOM až o 15 % v černici, 5 % v kambizemi a 3 % v černozemi, což bylo přičítáno stimulaci mikrobiální aktivity, která vedla k intenzivnějšímu rozkladu SOM.

Navzdory řadě hypotéz však zatím chybí cílené a komplexní studie, které by podrobně popsaly změny půdního mikrobiomu v souvislosti s přítomností P3HB. Současné poznatky však naznačují, že MP P3HB přesměrovávají půdní toky z ukládání C, N a dalších živin do půdní organické hmoty směrem k jejich zvýšené spotřebě, což má zásadní dopad na dostupnost N a celkovou funkčnost půdního ekosystému (Brtnicky et al., 2022).

2.6.7 Vliv P3HB na růst rostlin a rostlinnou biomasu

Nerovnováha živin v půdě vyvolaná biodegradací P3HB může vést ke snížení primární produkce rostlin v důsledku konkurence o zdroje mezi rostlinami, půdními mikroorganismy a rhizobiotou (Brtnický et al., 2024b) díky imobilizaci makroživin, což negativně ovlivňuje růst rostlin (Reay et al., 2024). To bylo potvrzeno na pro růst salátu napříč bez ohledu na půdní texturu (Brtnický et al. 2022), rajčata (Serrano-Ruiz et al. 2023), kukuřice (Brown et al. 2023), a pšenice (Zhou et al., 2021b). Autoři studie Malik et al. (2015) navíc uvedli, že akumulace P3HB v semenech transgenní rostliny *Camelina sativa* negativně ovlivnila klíčení, vzcházení i přežívání semenáčků. Fytotoxický účinek P3HB na vodní rostliny však doposud nebyl prokázán (Prochazkova et al., 2023).

Přestože kyselina 3-hydroxymáselná (3-HB), hlavní degradační produkt P3HB, má pKa 4,41 a tedy její uvolňování nevede přímo k silné acidifikaci (Bruss et al., 2008), může být působení kyseliny zesíleno přídavkem NPK hnojiv. P3HB zároveň ovlivňuje cyklus N v půdě v důsledku změn mikrobiální aktivity, diverzity a metabolismu, což má dopad na výživu a růst rostlin. Vedle přímého vlivu na růst rostlin se ukazuje, že degradační produkty P3HB mohou také ovlivňovat molekulární procesy v rostlinných buňkách. Bylo prokázáno, že kyselina 3-HB hraje významnou signální roli v regulaci eukaryotických buněk (Puchalska a Crawford, 2017). Mierziak et al. (2020) zjistili, že exogenní aplikace 3-HB u lnu (*Linum usitatissimum L.*) ovlivnila vzorce DNA de-/metylace a tím potenciálně i expresi genů spojených s fenylpropanoidní drahou. Tato dráha, mimo jiné, hraje důležitou roli v inaktivaci reaktivních forem kyslíku a odpovědi na abiotické stresy (Sharma et al., 2019). Nedávná studie také ukázala, že PHB vystavený zvětrávání v terénu vykazuje silnější inhibiční účinek na růst rostlin než čerstvý, dosud nerozložený bioplast (Serrano-Ruiz et al., 2023).

Tyto výsledky zdůrazňují potřebu dalšího výzkumu zaměřeného na pochopení mechanizmů účinku PHA v půdě, identifikaci ovlivňujících faktorů a vývoj strategií pro řízenou aplikaci a biodegradaci těchto materiálů v zemědělství.

3 CÍLE PRÁCE

Jak vyplývá z literární rešerše, MP z nebiodegradovatelných plastů ovlivňují jak vlastnosti půdy, tak i růst rostlin. Ačkoliv mají biodegradabilní plasty těmto negativním efektům plastů zabránit, prvotní experimenty naznačují, že způsobují nejen podobné problémy jako nebiodegradovatelné MP (změna pH, vliv na biodiverzitu), ale i řadu problémů nových. Cílem této práce bylo primárně ozřejmit vliv na produkční a mimoprodukční vlastnosti půdy, nicméně během řešení této práce se objevila i další témata, na která se tato práce pokouší alespoň částečně odpovědět. Dále je třeba také dodat, že tato práce rozvíjí přecházející disertační práci Dr. Fojta „Studium degradačních procesů bioplastů“, která byla zaměřena na studium procesů biodegradace biodegradabilních plastů a analýzu jejich reziduí v půdě. Zmíněná práce se také dotkla tématu týkajícího se vlivu biodegradace P3HB na růst rostlin a prolíná se s tématem této disertační práce publikací „Effect of biodegradablepoly-3-hydroxybutyrate amendment on the soil biochemical properties and fertility under varying sand loads“ publikovanou v roce 2020 v časopise Chemical and Biological Technologies in Agriculture. Na této publikaci Dr. Fojt spolupracoval, předložená disertační práce pak celé téma biodegradace P3HB rozvíjí a prohlubuje.

Jak je patrné z literární rešerše týkající se P3HB, dosavadní informace o vlivu PHB a jejich MP na kvalitu půdy a růst rostlin jsou oproti konvenčním MP stále neporovnatelně menší, i když se situace v posledních letech postupně mění (což mimo jiné podtrhuje i aktuálnost zvoleného tématu). Většina prvotních studií se zaměřila především na biodegradovatelnost P3HB jako takového, a proto v půdách bez rostoucí rostliny. To přineslo celou řadu originálních poznatků o vlivu půdních podmínek na biodegradaci jako takovou. Nicméně rhizosféra je unikátní a svojí podstatou se velmi liší od okolní půdy. Interakce mezi rostlinami a mikroby ovlivněnými nadbytkem labilního substrátu jsou proto zásadní pro pochopení environmentálních rizik spojených s využitím P3HB, obzvlášť vezmeme-li v úvahu fakt, že celá řada rhizosferních mikroorganismů má enzymový aparát umožňující metabolizaci P3HB. Dále pak půdní mikrobiom rychle reaguje na dostupnost uhlíku, což vede k šíření degradérů, kteří významně ovlivňují strategie rostlin a tím i rhizobiom. Jak vyplývá z literární rešerše, touto problematikou se již zabývalo několik studií, nicméně některé otázky zůstávají stále nezodpovězené. Jde především o to, zda a jak míra biodegradace, podíl zbytků bioplastů a aktivita degradátorů souvisí s konkrétními parametry půdy, toky živin a jejich příjmem rostlinami. Nejasná zůstávají i některá témata týkající se kvality půdní organické hmoty kontaminované P3HB a také hodnoty kritické koncentrace P3HB v půdě. Na základě výše uvedených úvah bylo definováno několik významných souhrnných obsahových výstupů práce zastřešujících odlišné tematické celky, které jsou shrnuty v pracích již publikovaných anebo v práci zaslané k recenznímu řízení (viz dále příloha této disertační práce). Konkrétně se jedná o následující tematické celky, které disertační práce objasňuje:

1. Vliv přídavku P3HB na dynamiku živin v půdě a kvalitu půdní organické hmoty ve vztahu ke změně půdního mikrobiomu.
2. Vliv změny ve stechiometrii živin v půdě na růst rostlin a tok živin do rostliny.
3. Vliv půdního prostředí na biodegradaci P3HB.
4. Možnosti snížení negativních účinků biodegradace P3HB na růst rostlin.

Práce, na něž je odkazováno jsou řazeny následovně:

1. Brtnicky, M., Kucerik, J., Skarpa, P., Mustafa, A., Siddiqui, M.H., Hammerschmiedt, T., Naveed, M., Kintl, A., Baltazar, T., Holatko, J., 2025. Dose-dependent effects of poly-3-hydroxybutyrate on soil quality and maize development: A trade-off between soil quality and crop productivity. Ecotoxicol. Environ. Saf. 295, 118131. https://doi.org/10.1016/j.ecoenv.2025.118131
2. Brtnicky, M., Holatko, J., Hammerschmiedt, T., Mustafa, A., Kamenikova, E., Kintl, A., Radziemska, M., Baltazar, T., Malicek, O., Kucerik, J., 2024. Effect of stabilized organic amendments on biodegradability of poly-3-hydroxybutyrate, soil biological properties, and plant biomass. Int. J. Environ. Sci. Technol. https://doi.org/10.1007/s13762-024-06061-1
3. Brtnicky, M., Pecina, V., Kucerik, J., Hammerschmiedt, T., Mustafa, A., Kintl, A., Sera, J., Koutny, M., Baltazar, T., Holatko, J., 2024. Biodegradation of poly-3-hydroxybutyrate after soil inoculation with microbial consortium: Soil microbiome and plant responses to the changed environment. Sci. Total Environ. 946, 174328. https://doi.org/10.1016/j.scitotenv.2024.174328
4. Brtnicky, M., Pecina, V., Holatko, J., Hammerschmiedt, T., Mustafa, A., Kintl, A., Fojt, J., Baltazar, T., Kucerik, J., 2022. Effect of biodegradable poly-3-hydroxybutyrate amendment on the soil biochemical properties and fertility under varying sand loads. Chem. Biol. Technol. Agric. 9, 75. https://doi.org/10.1186/s40538-022-00345-9
5. Brtnicky M., Mustafa A., Holatko J., Gunina A., Ondrasek G., Naveed M., Hammerschmiedt T., Kamenikova E., Alamri S., Siddique M.H., Kintl A., Baltazar T., Malicek O., Kucerik J. (2025): Soil texture-driven modulation of poly-3-hydroxybutyrate (P3HB) biodegradation: Microbial shifts, and trade-offs between

nutrient availability and lettuce growth, Environmental Research, Volume 278, 2025, 121618, ISSN 0013-9351. https://doi.org/10.1016/j.envres.2025.121618

6. Brtnicky M., Holatko J., Koutny M., Kucerik J., Hammerschmiedt T., Baltazar T., Sera J., Kintl A., Pecina V. Biodegradable microplastics impact on soil: How poly-3-hydroxybutyrate alters microbial diversity and nitrogen mineralization processes. Zasláno do časopisu Chemical and Biological Technologies in Agriculture.

Dále se pak téma biodegradace P3HB a vliv na půdu diskutuje ve třech dalších pracích, jichž je autor disertační práce spoluautorem. Tyto práce netvoří základ této disertační práce, a proto nejsou uvedeny v příloze, nicméně téma s nimi souvisí. Jedná se o následující práce, na něž je v některých částech diskuze odkazováno:

1. Fojt, J., Denková, P., Brtnický, M., Holátko, J., Řezáčová, V., Pecina, V., Kučerík, J., 2022. Influence of Poly-3-hydroxybutyrate Micro-Bioplastics and Polyethylene Terephthalate Microplastics on the Soil Organic Matter Structure and Soil Water Properties. Environ. Sci. Technol. 56, 10732–10742. https://doi.org/10.1021/acs.est.2c01970
2. Palucha, N., Fojt, J., Holátko, J., Hammerschmiedt, T., Kintl, A., Brtnický, M., Řezáčová, V., De Winterb, K., Uitterhaegen, E., Kučerík, J., 2024. Does poly-3-hydroxybutyrate biodegradation affect the quality of soil organic matter? Chemosphere 352, 141300. https://doi.org/10.1016/j.chemosphere.2024.141300
3. Trojan, M., Koutný, M., Brtnický, M., Holátko, J., Zlámalová Gargošová, H., Fojt, J., Procházková, P., Kalčíková, G., Kučerík, J., 2024. The Interaction of Microplastics and Microbioplastics with Soil and a Comparison of Their Potential to Spread Pathogens. Appl. Sci. 14, 4643. https://doi.org/10.3390/app14114643

4 VÝSLEDKY A DISKUZE

4.1 Vliv přídavku P3HB na dynamiku živin v půdě a kvalitu půdní organické hmoty ve vztahu ke změně půdního mikrobiomu

Dynamika živin v půdě a kvalita půdní organické hmoty úzce souvisí s aktivitou a složením půdního mikrobiomu. Mikroorganismy hrají klíčovou roli v koloběhu základních živin (hlavně C, N, P) a jejich dostupnost rostlinám často závisí na mikrobiomem zprostředkovaných procesech, jako je mineralizace, nitrifikace či fixace N. Kvalita půdní organické hmoty, zejména její chemické složení a poměr a obsah labilní a stabilní organické hmoty, ovlivňuje nejen rychlost mikrobiálního rozkladu, ale i strukturu mikrobiálních společenstev. Stabilnější formy organické hmoty podporují dlouhodobou akumulaci uhlíku v půdě (především ve formě organo-jílových komplexů), zatímco snadno rozložitelné látky stimulují mikrobiální aktivitu a krátkodobou mineralizaci živin. Interakce mezi půdní organickou hmotou a mikrobiálními procesy tak určují jak efektivitu cyklování živin, tak i celkovou půdní úrodnost a ekologickou stabilitu agroekosystémů.

Na otázku týkající se vlivu přídavku P3HB na dynamiku živin v půdě a kvalitu půdní organické hmoty ve vztahu ke změně půdního mikrobiomu je možné odpovědět těmito závěry:

Dynamika živin v půdě byla ovlivněna zvýšenou mikrobiální aktivitou při rozkladu P3HB výrazně ovlivňující koloběh živin, zejména C, N, P a S. Studie ukázaly, že P3HB stimuluje enzymy jako dehydrogenázu (DHA), ureázu (Ure), fosfatázu (Phos), N-acetyl-glukosaminidázu (NAG) a arylsulfatázu (ARS). DHA je enzym indikující celkovou mikrobiální aktivitu, protože se podílí na redoxních reakcích při buněčném dýchání a vzhledem k energetické náročnosti tvorby tohoto enzymu jeho zvýšená hladina naznačuje degradaci spíše komplexnějších organických struktur. Ure je produkována jak půdními mikroorganismy, tak i rostlinami, katalyzuje přeměnu močoviny na amoniak a oxid uhličitý a naznačuje tedy zvýšenou potřebu N. Podobně pak Phos, která uvolňuje anorganický P z organických sloučenin, což je klíčové pro dostupnost P rostlinám. NAG je enzym rozkládající chitin, čímž přispívá k uvolňování N z organické hmoty v půdě. Chitin tvoří stěny hub, exoskelety hmyzu a dalších půdních organismů, NAG tedy představuje indikátor mikrobiálního metabolismu N a rozkladu organické hmoty. Podobně ARS, která štěpí organické sloučeniny obsahující S a podílí se tedy na jejím cyklu v půdě. Zvýšená aktivita těchto enzymů proto svědčí o intenzivní mineralizaci těchto prvků, o zvýšeném toku živin a vychýlení přirozených mechanismů z rovnováhy.

Poměrně zajímavý je v tomto ohledu nárůst DHA, která funguje jako bioindikátor rovnováhy mezi akumulací a mineralizací SOM. Jeho vysoká aktivita může být výhodná v produkčních systémech s dostatkem čerstvé organické hmoty, ale ve zranitelných půdách bez obnovy organických vstupů může vést k degradaci půdní kvality. DHA je často využívána jako ukazatel celkové mikrobiální aktivity v půdě, protože dehydrogenázy jsou intracelulární enzymy zapojené do dýchacího řetězce mikroorganismů. Tyto enzymy katalyzují redoxní reakce, při nichž mikroorganismy přenášejí elektrony z organických substrátů na akceptory elektronů (např. kyslík nebo dusičnany), což je základní proces pro rozklad organické hmoty a uvolňování energie. Biodegradace P3HB mění strukturu SOM, v některých případech zvyšuje podíl stabilizovaných frakcí, ale zároveň může vést ke zrychlené mineralizaci původní SOM vlivem intenzivní mikrobiální aktivity. Tato „C-mining“ strategie vede k degradaci kvalitativně cenné SOM, což může dlouhodobě snižovat půdní úrodnost. Zde je tedy určité riziko, pokud se P3HB rozkládá v půdním prostředí, které je narušené. V této souvislosti se uvádí, že v České republice je více než 50 % půd ve velmi špatném stavu, celosvětově pak přibližně 33 % (VÚMOP, FAO). Poškozené půdy mají tendenci podléhat intenzivněji pozitivnímu priming efektu (proces spojený s degradací organické hmoty), podobně jak ukazuje práce Palucha et al. (2024).

Na téma P3HB v půdě a pozitivní priming efekt může zaznít námitka, že se jedná o polymer, který je syntetizován půdními organismy, a proto by měly priming efekt způsobovat i ostatní přírodní polymery, které se běžně v půdě vyskytují. Ve skutečnosti tomu tak opravdu je, pozitivní priming efekt byl pozorován i u dalších čistých látek jako jsou glukóza, fruktóza, alanin, celulóza, škrob a dokonce i u některých rostlinných zbytků (např. sláma) (Blagodatskaya et al., 2014; Blagodatskaya and Kuzyakov, 2011; Conde et al., 2005; Hamer and Marschner, 2005). U priming efektu způsobeného P3HB (a obecně čistými látkami) se jedná především o otázku množství P3HB, které se do půdy dostane, o rychlost, s jakou je plast degradován, a v neposlední řadě i o využitelnost C, tj. zda je P3HB využito více pro respiraci nebo pro tvorbu biomasy (z té pak stává nekromasa, která buď přispívá k tvorbě stabilních struktur, nebo je dále metabolizována).

Na druhou stranu se také ukázalo, že v krátkodobém horizontu může P3HB nahradit půdní organickou hmotu jako C-zdroj, čímž dochází k přesunu mikrobiální aktivity z přirozených substrátů na samotný polymer. Toto bylo pozorováno především u půdy s vysokým obsahem písku (nízkým obsahem organické hmoty), které jsou obvykle limitovány na většinu živin včetně C. P3HB jako labilní substrát mění směr mikrobiálního metabolismu, na jednu stranu stimuluje rozklad původní SOM (priming efekt, viz výše), ale zároveň přináší nový C, který může dočasně zvýšit celkový obsah C v půdě.

Velmi zajímavé jsou výsledky pro substrátem indukované respirace u půd kontaminovaných P3HB. V mnoha případech, po přídavku jiného labilního C-substrátu (glukóza, trehalóza), došlo k nárůstu respirační aktivity oproti kontrole, přičemž zvýšení také vykazovala i bazální respirace. To ukazuje paradoxní situaci, že i přes evidentní přebytek labilního C jsou stále v půdě mikroorganismy, které postrádají zdroj C a jsou patrně v dormantním stavu.

Rychlost rozkladu, ale především povaha P3HB, způsobuje změnu složení mikrobiálních společenstev. Přídavek negativně ovlivnil některé bakteriální rodiny např. *Nitrososphaeraceae, Xanthobacteriaceae,* což narušuje procesy nitrifikace a celkovou mineralizaci N, v důsledku čehož došlo k poklesu dostupného NO_3^--N v půdě a inhibici růstu rostlin. Podobně pak pokles *Gaiellaceae* může souviset se snížením dostupného fosforu (P). Naopak *Oxalobacteraceae, Sphingomonadaceae a Comamonadaceae* byly jeho působením stimulovány (většina jejich zástupců jsou nepatogenní organismy, nicméně některé mohou být potenciálně patogenní, např. *Acidovorax avenae* způsobuje choroby trav a rýže). Přirozeně narůstá podíl P3HB-degradujících taxonů (např. *Actinobacteria, Tetracladium*). Houbové společenstvo reagovalo výrazněji již při nízké dávce P3HB. Saprotrofní houby *Exophiala* a *Tetracladium*, které se podílejí na rozkladu organické hmoty, byly silně stimulovány, a naopak relativní zastoupení původně běžných rodů *Gibellulopsis a Fusarium* se výrazně snížilo. V některých variantách byly stimulovány i *Pseudeurotium a Cyberlindnera*. Reakce indexů biodiverzity ukázaly mírný pokles diverzity u variant s vyšší dávkou P3HB, což naznačuje ekologický stres a selektivní tlak.

4.2 Vliv změny ve stechiometrii živin v půdě na růst rostlin a tok živin do rostliny

Stechiometrie živin v půdě, tedy poměr mezi hlavními prvky (hlavně C, N a P), hraje klíčovou roli v regulaci růstu rostlin a toku živin do jejich pletiv. Rostliny mají specifické požadavky na poměry živin, a pokud je rovnováha narušena, může dojít k omezení příjmu některých prvků i při jejich dostatečném množství v půdě. Například příliš vysoký poměr C:N (přibližně nad 25) může zpomalit mineralizaci N, čímž se sníží jeho dostupnost pro rostliny. Naopak, nízký poměr N:P může signalizovat P limitaci, která omezuje růst kořenového systému, a tím i efektivitu příjmu ostatních živin.

Mikrobiální komunita v půdě reaguje na stechiometrii dostupných zdrojů a aktivně ovlivňuje koloběh živin. Pokud je například v půdě přebytek uhlíku, mikroorganismy intenzivně rostou a konkurují rostlinám o N a P, čímž může dojít k jejich dočasné imobilizaci. Vyvážená stechiometrie živin proto podporuje efektivní mikrobiální transformace a zajišťuje stálý tok živin směrem k rostlině. Tím je ovlivněna nejen rychlost růstu, ale i odolnost rostlin vůči stresu a schopnost využívat půdní zdroje v plném rozsahu. Například nadbytek N při nedostatku P může vést k neefektivnímu růstu biomasy bez odpovídající tvorby kořenového systému, což snižuje celkovou schopnost rostlin využívat dostupné zdroje.

Na otázku týkající se vlivu změny ve stechiometrii živin v půdě na růst rostlin a tok živin do rostliny se dá odpovědět takto:

V pracích se jednoznačně ukázalo, že problémem u variant s rostlinou oproti variantám bez rostliny je kompetice o živiny mezi mikroorganismy a pěstovanými rostlinami. Rychlá biodegradace P3HB podporuje růst mikroorganismů, které k metabolismu C potřebují zároveň i N a P, tím vzniká mikrobiální poptávka po N a P, která není uspokojena z polymeru samotného. Proto dochází ke krátkodobé imobilizaci živin, mikroorganismy si N a P „zabírají" pro sebe a omezují jejich dostupnost pro rostliny. Jako relativně bezpečná se zdá být dávka nepřesahující 0,1 %, i když jiní autoři uvádějí tento limit dokonce o řád nižší (Brown et al., 2023). Výsledkem je dříve diskutovaná mineralizace SOM a ztráta těchto klíčových živin nebo jejich forem využitelných rostlinami (zejména NO_3^--N a ortofosfátů). V praktickém důsledku dochází k výraznému poklesu obsahu NO_3^--N v půdě a k inhibici procesů nitrifikace (úbytek *Nitrososphaeria*, *Nitrospiria*), což dále snižuje tok N k rostlinám. Rostliny tak trpí deficitem minerálního N, což se projevuje redukcí nadzemní i kořenové biomasy; u salátu i kukuřice byl pozorován pokles suché biomasy o 80–99 % a to i při relativně nízkých dávkách (1 %). Tento pokles byl nejvýraznější při vyšších dávkách (5–10 %) a v písčitějších půdách, které mají nižší pufrační a zásobní schopnosti. Růst rostlin nebyl podpořen ani inokulací půdy mikroorganismy schopnými fixovat N nebo solubilizovat P, což potvrzuje, že změna stechiometrie je natolik silná, že překonává potenciální přínos těchto mikrobiálních funkcí. Otázkou samozřejmě také zůstává, zda přídavek alochtonních organismů vyvolá takovou změnu v půdním mikrobiomu, která bude schopna živinovou nerovnováhu vyvážit.

Co se týká toku živin do rostliny, zásadním nutrientem se jeví N. Při biodegradaci dochází k jeho mikrobiální imobilizaci, větší část N je „uvězněna" v mikrobiální biomase a není dostupná pro kořeny rostlin. To vede ke stresu rostliny, ta se snaží nedostatek kompenzovat regulací kořenových exudátů a nárůstem aktivity enzymů jako je ureáza, nicméně ani zvýšená exudace nevede k vyšší dostupnosti pro rostliny. Nutno dodat, že je to mimo jiné i tím, že rostlina má i nedostatek dalších prvků a musí své potřeby prioritizovat (což vede ke změně složení kořenových exudátů).

Obsah draslíku (K) v půdě nebyl identifikován jako limitující faktor růstu rostlin, bylo zjištěno že růst salátu byl výrazněji ovlivněn nedostatkem N a P. Také nebyl zaznamenán pokles enzymů či fyzikálně-chemických ukazatelů, které by signalizovaly zhoršený tok K. Ostatní stopové prvky nebyly sledovány, nicméně je možné, že inhibice růstu rostlin může být způsobena nejen nedostatkem makroživin, ale i mikronutrienty, které nebyly analyzovány, ale jejich role by mohla být významná zvláště při vysoké mikrobiální konkurenci.

Přídavek P3HB a jeho následná mikrobiální degradace může ovlivnit pH půdy, a tím i celou řadu půdních procesů. Při rozkladu P3HB vznikají organické kyseliny (např. butyrát, acetát), které mohou lokálně snižovat pH a způsobit mírné okyselení půdního prostředí. Tento jev je zvlášť významný v půdách s nižší pufrační schopností (např. písčitějších), kde se pH sníží snadněji než v půdách s vyšším obsahem jílu a organické hmoty.

Změna pH má přímý dopad na dostupnost živin, např. P se stává méně dostupným při silně kyselém nebo zásaditém pH kvůli tvorbě nerozpustných forem. N ve formě amonného iontu (NH_4^+) je stabilnější při nižším pH, zatímco nitrifikace (přeměna NH_4^+ na NO_3^-) je omezena, protože nitrifikační bakterie (např. *Nitrososphaeria*) jsou na pH citlivé a jejich aktivita klesá v kyselém prostředí. To přispívá k pozorovanému poklesu nitrifikace a snížení obsahu NO_3^--N v půdě s P3HB. Současně došlo k nárůstu anaerobních taxonů, např. *Clostridium*, což může naznačovat změnu redoxních podmínek a nepřímo i pH (kyselé a anaerobní prostředí často jdou ruku v ruce při vysoké spotřebě O_2 mikroorganismy).

Byla pozorována změna poměru bakterie:houby. Houby byly v některých případech po přídavku P3HB více zastoupené (*Tetracladium, Exophiala* aj.), což může být dáno lepší tolerancí k nízkému pH. Nutno dodat, že z pohledu rostlin může okyselení rizosféry ovlivnit i kořenový růst, protože nízké pH omezuje příjem některých živin (např. P, Ca, Mg) a zároveň zvyšuje toxicitu jiných prvků (např. Al, Mn).

4.3 Vliv půdního prostředí na biodegradaci P3HB

Půdní prostředí má zásadní vliv na rychlost a účinnost biodegradace bioplastů, a to včetně P3HB. Klíčovými faktory, které ovlivňují rozklad těchto materiálů, jsou především teplota, vlhkost, pH, obsah organické hmoty, dostupnost živin a složení půdního mikrobiomu. Vyšší teplota a dostatečná vlhkost zpravidla urychlují aktivitu mikroorganismů zodpovědných za degradaci bioplastů, protože podporují růst bakterií a hub schopných produkovat specifické enzymy (např. PHB depolymerázy). Naopak extrémně suché, chladné nebo kyselé/alkalické podmínky mohou biodegradaci výrazně zpomalit.

Důležitá je také kvalita a množství půdní organické hmoty – půdy s vyšším obsahem rozložitelného C obecně podporují mikrobiální aktivitu, avšak mohou zároveň způsobit kompetici o živiny, čímž se biodegradace bioplastů oddálí. Kromě toho může být degradace ovlivněna i typem půdy – například jílovité půdy s vyšším obsahem sorpčně aktivních částic mohou zpomalovat přístup enzymů k povrchu bioplastu.

Složení a diverzita mikrobiální komunity hraje rovněž významnou roli. Půdy bohaté na specializované mikroorganismy (např. určité druhy *Bacillus, Pseudomonas* nebo *Streptomyces*) vykazují vyšší degradační potenciál. Celkově lze říci, že biodegradace bioplastů je velmi citlivá na podmínky půdního prostředí, a pochopení těchto interakcí je klíčové pro efektivní využití bioplastů v zemědělské praxi nebo při environmentálních aplikacích.

Během studia vlivu biodegradace P3HB na půdu bylo zaznamenáno několik zajímavých výsledků týkajících se témat, na které předcházející práce neodpověděly, nebo je jejich autoři nediskutovali.

Půdní textura výrazně ovlivňuje rychlost a průběh biodegradace. Písčité půdy mají nižší sorpční kapacitu a nižší aktivitu půdní biomasy, to vede k pomalejšímu rozkladu P3HB, ale současně méně mikrobiální konkurence o živiny. Půdy bohaté na organickou hmotu nebo jemné částice (jíly, hlína) podporují rychlejší kolonizaci polymeru mikroorganismy a vyšší aktivitu enzymů rozkládajících P3HB. Nicméně existuje určitá „optimální textura půdy", která zajišťuje lepší provzdušnění, patrně i distribuci a zádrž vody. To naznačuje, že v těžkých půdách může být degradace pomalejší než v lehčích, může dojít ke snižování kyslíkové dostupnosti a k vyšší mikrobiální kompetici.

Udržování optimální půdní vlhkosti (~60 % vodní kapacity) je klíčové, všechny experimenty s řízenou vlhkostí vedly k úspěšné degradaci. V suchých půdách (nebo při nedostatečné hydrataci půdy) se degradace výrazně zpomaluje. P3HB je slabě hydrofilní (Fojt et al., 2022), což omezuje kontakt s vodním filmem, degradace může být pomalejší bez dobrého zavlažování. Přílišná vlhkost však může vést k anaerobióze, která zpomaluje aerobní degradaci a může měnit spektrum degraderů (např. nárůst *Clostridium*). Výsledky naznačily, že v některých variantách P3HB stimuloval anaerobní mikroorganismy, což naznačuje změny v mikroprostředí (např. v mikroporech kolem plastu nebo plastisféry (Trojan et al., 2024).

Experimenty probíhaly při mírných teplotách (18–22 °C). Výsledky ukázaly, že i bez vysokých teplot lze dosáhnout účinné degradace P3HB, pokud jsou přítomny aktivní mikroorganismy a vlhkost. Vyšší teploty (např. v kompostu nebo letní polní podmínky) by degradaci pravděpodobně ještě urychlily. V publikovaných pracích nebyly testovány, nicméně jsou finalizovány další práce, v rámci jejichž experimentů se vliv zvýšené teploty v půdě jednoznačně projevil.

Původní půdní mikrobiom má zásadní význam, přítomnost bakterií rodu *Pseudomonas, Bacillus, Streptomyces, Azospirillum, Actinobacteria* nebo saprofytických hub (*Tetracladium*) výrazně zvyšuje degradační aktivitu. Inokulace mikrobiálním konsorciem (PGPR + N-fixující mikroorganismy) vedla ke zrychlení biodegradace (např. z 46 % na 65 % u 1 % dávky P3HB za 56 dní), zvýšení enzymatické aktivity (DHA, GLU, Phos aj.), nevedla ale k efektivnímu potlačení negativního vlivu P3HB na růst rostlin. Studie potvrdily, že při vyšším podílu písku dochází k relativnímu nárůstu poměru houby vs. bakterie, což může změnit profil degradace.

Jak již bylo zmíněno dříve, P3HB může mírně okyselit půdu díky vzniku 3- hydroxybutyrátu, ale ve studiích bylo potvrzeno, že při pH neutrální půdě (okolo 7,3) to není limitující faktor degradace. Nicméně v kyselých půdách by toto okyselení mohlo ovlivnit aktivitu mikroorganismů i růst rostlin (ačkoliv některé houby jsou schopny degradovat P3HB i za kyselejších podmínek).

4.4 Možnosti snížení negativních účinků biodegradace P3HB na růst rostlin

Biodegradace labilních substrátů v půdě, v tomto případě P3HB, se ukázala jako problematická pro růst rostlin. Nicméně labilní substráty (cukry, organické kyseliny), mohou mít i pozitivní účinky. Je to dáno především zvýšením mikrobiální aktivity a mineralizace živin, protože labilní substráty slouží jako rychlý zdroj energie pro půdní mikroorganismy, což podporuje jejich růst a činnost. Aktivní mikrobiální komunita následně rozkládá složitější organické látky a uvolňuje živiny (zejména N a P), které jsou pak dostupné rostlinám.

Další aspektem je zlepšení struktury půdy, produkty mikrobiální aktivity, jako jsou exopolysacharidy, mohou zlepšit agregaci půdy, což přispívá k lepšímu provzdušnění, vodnímu režimu a tím i podpoře kořenového růstu. Nakonec i vytvořená nekromasa přispívá k ukládání organického C. V neposlední řadě je třeba zmínit i stimulaci rhizosférních interakcí, protože uvolňování labilních uhlíkatých látek může stimulovat symbiotické vztahy, např. s mykorrhizními houbami, což dále zlepšuje příjem živin.

Jak ukázaly výsledky, negativní vliv P3HB na růst rostlin se objevuje především kvůli kompetici mezi zvýšenou mikrobiální aktivitou způsobenou degradací P3HB a požadavky rostliny pro růst. Vzhledem k tomu, že se P3HB jeví jako zajímavý materiál v zemědělství, byla v rámci vybraných experimentů snaha daný proces zvrátit jak inokulací PGPR tak i některými amendmenty s následujícími výsledky:

Použití inokula obsahujícího *Azospirillum, Azotobacter, Bacillus, Pseudomonas* apod. mělo za cíl zvýšit fixaci N, podpořit mineralizaci P a urychlit samotnou degradaci P3HB. Výsledek ukázal, že inokulace zvýšila rychlost degradace P3HB i enzymatickou aktivitu půdy, nicméně již nezmírnila negativní dopad na růst rostlin, protože rostlinná biomasa zůstala snížená i při přítomnosti inokulantu. Tento typ inokulace se tedy ukázal jako nedostačující, mikroorganismy z aplikovaného inokula samy spotřebovávají živiny, což zvyšuje konkurenční boj o živiny, tj. o N a P. Při aplikaci inokula se ovšem nabízí již výše diskutovaná otázka o smysluplnosti využití alochtonních bakterií při podpoře procesů biodegradace a obecně při využití pro zvýšení úrodnosti. Celá řada autorů tuto strategii podporuje, jiní jsou skeptičtí vzhledem k tomu, že autochtonní organismy jsou vždy v obrovském přebytku a je jen otázkou času, kdy po inokulaci dojde k ustanovení původní mikrobiologické rovnováhy.

Další strategie pro potlačení negativních účinků biodegradace P3HB zahrnovala využití stabilizovaných organických substrátů, jakými jsou digestát a kompost, přičemž tyto amendmenty měly zajistit dodatečný N a P, které mikrobiální biomasa potřebuje pro růst. Výsledkem bylo zmírnění negativních účinků P3HB na růst rostlin, i když výsledky nedosáhly stejných hodnot růstu jako v kontrole bez P3HB. Nicméně kombinace P3HB s N- a P-bohatými organickými amendmenty, ale i hnojivy, představuje nadějnou strategii pro vyrovnání živinové bilance. V této souvislosti je třeba zmínit i to, že roli hraje patrně i původní substrát pro tvorbu těchto amendmentů. Dále lze předpokládat, že zvýšení dávek by proces biodegradace kompletně vyrovnalo.

Další možnosti, které se s ohledem na znalosti procesů biodegradace nabízejí, zahrnují optimalizaci půdního prostředí (pH, vlhkost, struktura), vzhledem k tomu, že neutrální pH, dostatečná vlhkost a bohatá organická hmota podporují zdravý mikrobiom i růst rostlin. V dobře strukturovaných a mírně jílovitých půdách byl dopad P3HB méně výrazný než v písčitých substrátech. Volba vhodného půdního typu a jeho úprava může tlumit negativní účinky.

Dalším faktorem může být aplikační strategie a načasování. Aplikace P3HB před výsadbou rostlin (např. s časovým předstihem několika týdnů) může umožnit počáteční degradaci a imobilizaci živin mikrobiomem a následně uvolnění živin zpět do půdy (mineralizace druhé vlny). Tato strategie zatím nebyla testována, ale může být relevantní hypotézou pro budoucí výzkum.

5 ZÁVĚR

P3HB má obrovský potenciál uplatnění v celé řadě odvětví, včetně zemědělství, kde díky jeho biodegradabilitě v kombinaci s dalšími vlastnostmi dochází k jeho narůstajícímu uplatnění. Je to právě rychlá biodegradabilita, která je podstatným přínosem pro tyto aplikace. V půdě, kde je určité množství přirozeně biosyntetizovaného P3HB, degraduje tento substrát za příznivých podmínek také poměrně rychle. Nicméně právě tato rychlost se zdá být faktorem limitujícím vstup většího množství P3HB do půdy. Tato situace je analogií výroku připisovaného Paracelsovi (Philippus Aureolus Theophrastus Bombastus von Hohenheim, 1492–1541) „Všechny sloučeniny jsou jedy. Neexistuje sloučenina, která by jedem nebyla. Rozdíl mezi lékem a jedem tvoří dávka.“ I zde se zdá, že větší množství P3HB v půdě (ať už pocházející z aplikace v zemědělství nebo v budoucnu i z jiných zdrojů), lze již považovat za problematické, a že je tedy možné označit P3HB za kontaminant, který narušuje půdní prostředí a posunuje jak živinovou, tak i mikrobiální rovnováhu.

Nicméně pochopení problému může také vést k jeho nápravě. Aplikací postupů, které práce naznačuje, lze tyto problémy nejen eliminovat, ale i využít ku prospěchu, například pro ukládání C v půdě, případně za účelem oživení půdního mikrobiomu. Na závěr lze tedy konstatovat, že využití P3HB v zemědělských aplikacích má svůj potenciál, je však třeba brát ohledy na některá rizika spojená s jeho použitím.

6 SEZNAM NEJČASTĚJI POUŽITÝCH ZKRATEK

AMF – arbuskulární mykorhizní houby

ARG – antirezistentních genů

DOM – vodorozpustný uhlík/rozpuštěná organická hmota

EBA – ethylen-butyl-akrylát

EVA – ethylen-vinylacetát

FDAse – fluorescein diacetát hydrolázy

HDPE – vysokohustotní polyethylen

HGT – horizontální přenos genů

HOC – hydrofobních organických chemických látek

LLDPE – lineární nízkohustotní polyethylen

MBP – mikrobioplasty

MP – mikroplasty

NIR – blízké infračervené záření

NP – nanoplasty

PA – polyamid

PAH – polycyklické aromatické uhlovodíky

PBAT – polybutylen adipát-ko-tereftalát

PBS – polybutylensukcinát

PC – polykarbonát

PCB – polychlorované bifenyly

PCL – polykaprolakton

PE – polyethylen

PET – polyethylentereftalát

pH – půdní reakce

PHA – polyhydroxyalkanoát

PHB – polyhydroxybutyrát

P3HB – poly-3-hydroxybutyrát

PLA – kyselina polymléčná

PMMA – polymetylmetakrylát

POP – perzistentní organický polutant

PP – polypropylen

PS – polystyren

PVC – polyvinylchlorid

SOM – půdní organická hmota

7 SEZNAM POUŽITÝCH ZDROJŮ

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Dose-dependent effects of poly-3-hydroxybutyrate on soil quality and maize development: A trade-off between soil quality and crop productivity

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ABSTRACT

Poly-3-hydroxybutyrate (P3HB), a biopolymer synthesized by soil bacteria, has emerged as a promising tool for sustainable agriculture, offering dual benefits as a carbon reservoir and an eco-friendly biotechnological product. However, its impact on soil nutrient dynamics and plant nutrient uptake remains underexplored. This study evaluated the effects of P3HB biodegradation on soil properties and maize (*Zea mays*) growth in a pot experiment with five P3HB application rates (0–10 % w/w), in both planted and unplanted soils. Key analyses included soil pH, enzyme activity, microbial biomass carbon (MBC), nutrient contents in soil and plant biomass, and residual P3HB (a rarely addressed aspect in previous research). The addition of P3HB influenced soil biota in both planted and unplanted soils, showing consistent trends across application rates. P3HB reduced soil pH (from 7.4 to 7.1 at 1 % and 6.4 at 10 % P3HB in unplanted soil) and increased total carbon (by approximately 100 % in unplanted and 65 % in planted soils at 10 % P3HB). In unplanted soils, P3HB degraded more quickly, but enzyme activities of β-glucosidase and phosphatase decreased by 20 % and 15 %, respectively. Conversely, arylsulphatase and urease activities increased by 80 % and 200 %, respectively, in both soil variants in both variants. Microbial biomass carbon increased by 500 % in unplanted soils compared to the unamended control, while planted soils showed a 10 % increase. Available nutrients (K and P) were higher in unplanted soils compared to planted soils. In planted soils, competition for nutrients (N, P, K) among maize plants, the rhizobiome, and P3HB-degrading microbes led to reduced above-ground biomass at higher P3HB application rates (from 5.6 g to 0.5 g per plant at 1 % P3HB). Statistical analysis (Eta-squared values and ANOVA) revealed that P3HB dose primarily influenced soil physico-chemical properties and plant parameters, whereas maize planting had a smaller impact, affecting only pH and MBC. P3HB biodegradation improved soil properties, particularly by increasing MBC and total carbon. However, application rates of 1 % and above caused slight acidification, increased nutrient competition, and reduced nutrient availability, ultimately hindering maize growth. These results underscore the trade-offs between improving soil quality and maintaining crop productivity, highlighting the importance of optimizing P3HB application rates in agricultural systems. This study provides critical insights into the dual effects of biodegradable plastics like P3HB, emphasizing their potential as microbial carbon storage polymers while cautioning against excessive use in crop production.

1. Introduction

Biodegradable polymers have emerged as a promising tool for sustainable agriculture, offering a viable alternative to conventional plastics and synthetic soil amendments (Trojan et al., 2024). Among biodegradable materials, polyesters are the most preferred candidates,

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represented by polymers such as polylactic acid (PLA), polycaprolactone (PCL), polybutylene succinate (PBS) (Ng et al., 2018). In recent years, polyhydroxyalkanoates (PHAs) have also gained significant attention due to beneficial properties such as biocompatibility, thermal processability, and satisfactory mechanical properties (Medeiros Garcia Alcantara et al., 2020).

Poly-3-hydroxybutyrate (P3HB), the most widely studied PHA, is synthesized by soil bacteria as a carbon and energy storage compound under conditions of nutrient imbalance (Alves et al., 2017). It is biodegradable in microbially active environments, including freshwater and seawater (Briassoulis et al., 2019), anaerobic digestion sludge (Cazaudehore et al., 2023), municipal sewage sludge (Gutierrez-Wing et al., 2010), sediment (Eich et al., 2021), soil (Serrano-Ruiz et al., 2023). These unique properties make P3HB an attractive candidate for agricultural applications, where it can serve as a carbon reservoir, improve soil structure, and support microbial activity.

The natural concentration of P3HB in soil typically varies and it is measured in milligrams of P3HB per gram of soil microbial carbon (Elhottová et al., 2000). In soil, P3HB can be utilized by a broad spectrum of microorganism, including saprophytic fungi (Altaee et al., 2016) and bacteria (Volova et al., 2017) and it represents a natural part of soil food web (White et al., 2021).

However, higher concentrations of P3HB introduced through external inputs are not harmless as once believed. Critical concerns regarding P3HB contamination in soil include its effects on pH, nutrient balance and availability (including oxygen levels), soil biochemistry and the excessive stimulation of microbial activity (Brtnicky et al., 2024b). P3HB degradation has been shown to both influence and be influenced by soil pH, as the release of 3-hydroxybutyrate (3-HB) during biodegradation can alter the pH environment (Volova et al., 1998). Additionally, P3HB has been reported to significantly enhance soil respiration and CO_2 emission (Liu et al., 2019), while also shifting microbial community composition to favor P3HB degraders (Fernandes et al., 2020). The accelerated rates of carbon mineralization driven by PHA microparticles in contaminated soil (Zhou et al., 2021b) have also been shown to enhance the turnover of other nutrients, particularly of soil nitrogen content (Brtnicky et al., 2022). Due to high carbon-to-nitrogen ratio, PHA alters the accessibility and utilization of indigenous organic matter by soil microbes (Pathan et al., 2020).

This nutrient imbalance also leads to a reduction in plant primary production caused by competition for resources among soil microorganisms, plants, and rhizobiota (Brtnicky et al., 2024b). For instance, it has been reported that field-weathered biodegradable mulch added to soil had strong detrimental effects on tomato plants and completely inhibited the development of lettuce (Serrano-Ruiz et al., 2023). Therefore, a stoichiometry imbalance caused by an excess of labile carbon negatively impacts the soil's biochemical cycles (Brown et al., 2023).

Despite increased awareness of the impact of biodegradable plastics on soil and plant growth, significant knowledge gaps remain regarding how P3HB influences microbial activity, nutrient dynamics and availability. First, most studies have focused on the biodegradation of P3HB in unplanted soils (Brown et al., 2023; Brtnicky et al., 2022), with limited attention to its effects in planted systems where plant-microbe interactions play a critical role. Second, these impacts are dose-dependent, with the soil-to-bioplastics ratio changing rapidly as biodegradation progresses. Soil microbiome response quickly to carbon availability, leading to the proliferation of degraders, which significantly influences plant and rhizobiome strategies. Although several studies have addressed aspects of this issue (Altaee et al., 2016; Brown et al., 2023; Brtnicky et al., 2022; Serrano-Ruiz et al., 2023; Volova et al., 2022; Zhou et al., 2021a), it remains unclear whether and how the degree of biodegradation, the proportion of bioplastic residues, and the activity of degraders are linked to specific soil parameters, nutrient flows, and nutrient uptake by plants. Third, the fate of residual P3HB in soil and its long-term effects on soil properties and plant growth have rarely been addressed.

The objectives of this work were to (i) assess the rate of P3HB degradation and its impact on soil organic carbon and microbial biomass carbon across varying application rates in both unplanted and maize-planted soil and, (ii) evaluate the effects of P3HB amendment on nutrient availability and maize biomass, exploring potential competitive interactions between plant roots and soil microorganisms, and (iii) establish links between soil and plant properties to residual P3HB and determine the optimal rate for soil quality and crop productivity.

2. Materials and methods

2.1. Soil preparation and properties

The soil used in this work was sampled from a depth of 0–15 cm near the town of Troubsko, Czech Republic (49°10'28"N 16°29'32"E) and classified as silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification). Soil was air dried and sieved through 2 mm mesh sieve. Its chemical properties were determined by Mehlich 3 method giving total C (14.0 $g \cdot kg^{-1}$), total N (1.60 $g \cdot kg^{-1}$), available P (0.10 $g \cdot kg^{-1}$), Ca (3.26 $g \cdot kg^{-1}$), Mg (0.24 $g \cdot kg^{-1}$), and K (0.23 $g \cdot kg^{-1}$). The initial pH ($CaCl_2$) of the soil was 7.3.

2.2. Procurement and properties of poly-3-hydroxybutare

P3HB was obtained in powder form (< 80 μm) from TianAn Biologic Materials Co., Ltd. (Ningbo City, China) under the trade name ENMAT Y3000. The contact angle of P3HB has previously been reported between 70° and 81° and the crystallinity was around 49 % (Procházková et al., 2024). Further information on P3HB characters can be found in (Fojt et al., 2022). P3HB without additives was used to avoid the influence of other chemicals as their content in plastics can be as high as 70 % (Steinmetz et al., 2016).

2.3. Experimental design and treatments

The pot experiments testing the impact of P3HB on plant growth were conducted in a growth chamber at Mendel University in Brno, Czech Republic. The growth substrates used for the pot experiment were prepared by mixing soil with P3HB powder at five weight concentrations: 0 %, 0.1 %, 1 %, 5 %, 10 %. This loading rate was chosen based on previous studies. Specifically, the range was explained by (Brown et al., 2023) as representing scenarios such as "homogenous mixing in soil," "heterogenous mixing in soil," "plastic contamination hotspots in soil," and "field plastic waste dump sites."

The experimental variants are outlined in Table 1. Specifically, plastic pots with a capacity of 2 L were filled with 1.7 kg of soil (negative control) or the same amount of mixtures of soil and P3HB. No fertilizers were applied to the pots. Each P3HB treatment was prepared in 10 replicates. These replicates were then divided into two groups of five pots: one group was sown with maize (*Zea mays L.*), while the other remained unplanted.

All experimental pots, both sown and unplanted, were watered with 300 mL of distilled water and randomly placed in a growth chamber (CLF Plant Climatics GmbH, Germany) under controlled conditions. The chamber settings included a 12-hour photoperiod, light intensity of 20,000 lx, air temperatures of 20°C during the day and 12°C at night, relative humidity of 45 % during the day and 70 % at night, and soil moisture maintained at 65 % of the water-holding capacity (WHC) by watering every other day. Ten days after seedling emergence, the number of seedlings per pot was reduced to the two largest ones.

2.4. Plant analyses

After 90 days, plants were harvested at ground level, and their height was measured. The biomass from each pot was then dried at 60 °C to a

Table 1
Experimental variants amended with various doses of poly-3-hydroxybutyrate, planted or unplanted with maize.

Amendment variant	**Doses per 2-L pot**				Variant	Replication
	Soil [g]	P3HB [g]	maize (*Zea mays* L.)			
			seeds	plants		
0 % P3HB (negative control)	1700	0	0	0	unplanted	5
			6	2	maize-planted	5
0.1 % P3HB	1698.3	1.7	0	0	unplanted	5
			6	2	maize-planted	5
1 % P3HB	1683	17	0	0	unplanted	5
			6	2	maize-planted	5
5 % P3HB	1615	85	0	0	unplanted	5
			6	2	maize-planted	5
10 % P3HB	1530	170	0	0	unplanted	5
			6	2	maize-planted	5

constant weight to determine above-ground biomass (AGB_dry). Dry plant samples were prepared for elemental analysis. Kjeldahl digestion procedure was used for total nitrogen (N) content, while for phosphorus (P) and potassium (K) content determination was used the microwave digestion using an ETHOS 1 instrument (Milestone Srl, Sorisole, Italy). The extracts were analyzed by atomic absorption spectrometry (AAS) using the system Agilent 55B AA (Agilent Technologies, Santa Clara, CA, USA).

2.5. Soil analyses

A mixed soil sample was taken from each pot after harvesting the maize, a part was air dried and a part was stored at 4°C for microbial carbon analysis. Air-dried soil samples were analyzed for pH (ISO_10390, 2005) and basic soil nutrients. TC and TN were measured using the elementar analyser Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Available P and K were analyzed after extraction using Mehlich 3 extraction procedure and analyzed by using AAS. Freeze-dried samples were used for the analysis of enzymatic activities (ISO_20130, 2018): β-glucosidase (GLU), arylsulfatase (ARS), phosphatase (Phos), and urease (Ure). The *p*-nitrophenol (PNP) derivatives of the specific soil substrates were measured using a Vis spectrophotometric measurement (Infinite M Nano, Tecan Trading AG, Switzerland) at 405 nm (β-glucosidase, arylsulfatase and phosphatase). Urease activity was determined as the amount of ammonium produced from the substrate urea, detected spectrophotometrically using the cyanurate reagent (at 650 nm). Each soil sample was measured in nine replicates. Samples stored at 4 °C were used for determining microbial biomass carbon using the fumigation extraction method (Vance et al., 1987).

2.6. Thermogravimetric analysis of P3HB residues

P3HB residues were determined using thermogravimetry according to procedure described in (Palucha et al., 2024). Briefly, soil samples were analyzed using a thermogravimeter TGA 550 (TA Instruments, New Castle, Delaware, USA). Approximately 200 mg of soil samples, previously equilibrated for two weeks for equilibrium moisture, were heated on an alumina pan from 25 to 700°C, at heating rate of 5 °C per minute, under a dynamic air atmosphere (90 mL min^{-1}). The analyses were conducted for all pots, soil in each pot was analyzed in triplicate.

The TG data served for following calculations:

1. Determination of P3HB residues: the P3HB thermally degrades between 200 and 300°C, therefore mass losses obtained in this temperature interval for control soil samples (without P3HB) were subtracted from respective P3HB-amended variants as demonstrated previously by (Palucha et al., 2024). Mass loss corresponded to the residual P3HB in respective variant.
2. Carbon content originating from residual P3HB: the residual P3HB contained carbon, which contributed to the total carbon (TC) content in soil. To separate these two carbon types, the carbon content in residual P3HB was calculated. Specifically, a monomer unit of P3HB has a molecular weight of 85 g mol^{-1} and contains four carbon atoms, which constitutes of 56 % of the monomer unit. Consequently, this value was subtracted from the total carbon determined using elemental analysis to isolate the soil organic carbon without P3HB residues. In this way were obtained TC_corr and consequently TC_corr/N. The potential contribution from maize root exudates was neglected in calculation, as the carbon content in planted and unplanted variant did not differ significantly (Fig. 1C).
3. The carbon in SOC originating from P3HB (P3HB related carbon) for each amended variant was calculated by the subtraction of the corrected SOC in the control soil from the SOC in the P3HB-amended soils.

2.7. Statistical analysis

Data processing and statistical analyses were performed using software R, version 4.3.2. (R_Core_Team, 2023). One-way ANOVA (for plant properties) and two-way ANOVA (for all other properties) were performed using a type I (sequential) sum of squares at a 0.05 significance level (Zar, 1984). The two-way ANOVA examined changes in quantitative dependent variables based on two categorical independent variables: "P3HB dose" and "Plant." The analyses were conducted using the "FactoMineR" (Lê et al., 2008) and "factoextra" (Kassambara and Mundt, 2020) packages The size of factor effect on difference among variables was carried out with help of partial eta-squared (η_p^2) from package „lsr" (Navarro, 2015). Tukey's honestly significant difference (HSD) test following ANOVA from package „agricolae" (de Mendiburu, 2023) was used for calculating factor level means, also at a significance level of 0.05. "Treatment contrast" was applied to calculate the mean with SEM for each treatment. Package "ggplot2" (Wickham, 2016) was used for creating graphs. Pearson's correlation analysis was performed to measure the linear dependence between soil properties. Correlation coefficient (r) was interpreted as follows: $0.0 < r < 0.3$ (negligible correlation), $0.3 < r < 0.5$ (low correlation), $0.5 < r < 0.7$ (moderate correlation), $0.7 < r < 0.9$ (strong correlation), $0.9 < r < 1.0$ (very strong correlation) (Hinkle et al., 2003).

3. Results

3.1. Soil chemical properties

Fig. 1 illustrates the impact of P3HB amendments on various soil physico-chemical properties. Fig. 1A shows that soil pH remained relatively stable across different P3HB treatment levels, with minor variations among doses. Maize-planted soils tended to have slightly higher pH values compared to unplanted soils at all P3HB levels. A slight decrease in pH was observed with increasing P3HB concentrations. The microbial biomass carbon (MBC), depicted in Fig. 1B showed a marked increase with higher P3HB doses, especially at 5 % and 10 %, where maize-planted soils consistently exhibited higher MBC values than unplanted soils. Significant increases were observed among P3HB doses, with MBC progressively rising from the lowest to the highest P3HB concentration. Total carbon (TC), which includes also residues of P3HB (Fig. 1C), also increased significantly at higher P3HB levels, with the highest values observed at 10 % P3HB, regardless of planting conditions. Maize-planted soils generally showed slightly higher TC levels compared to

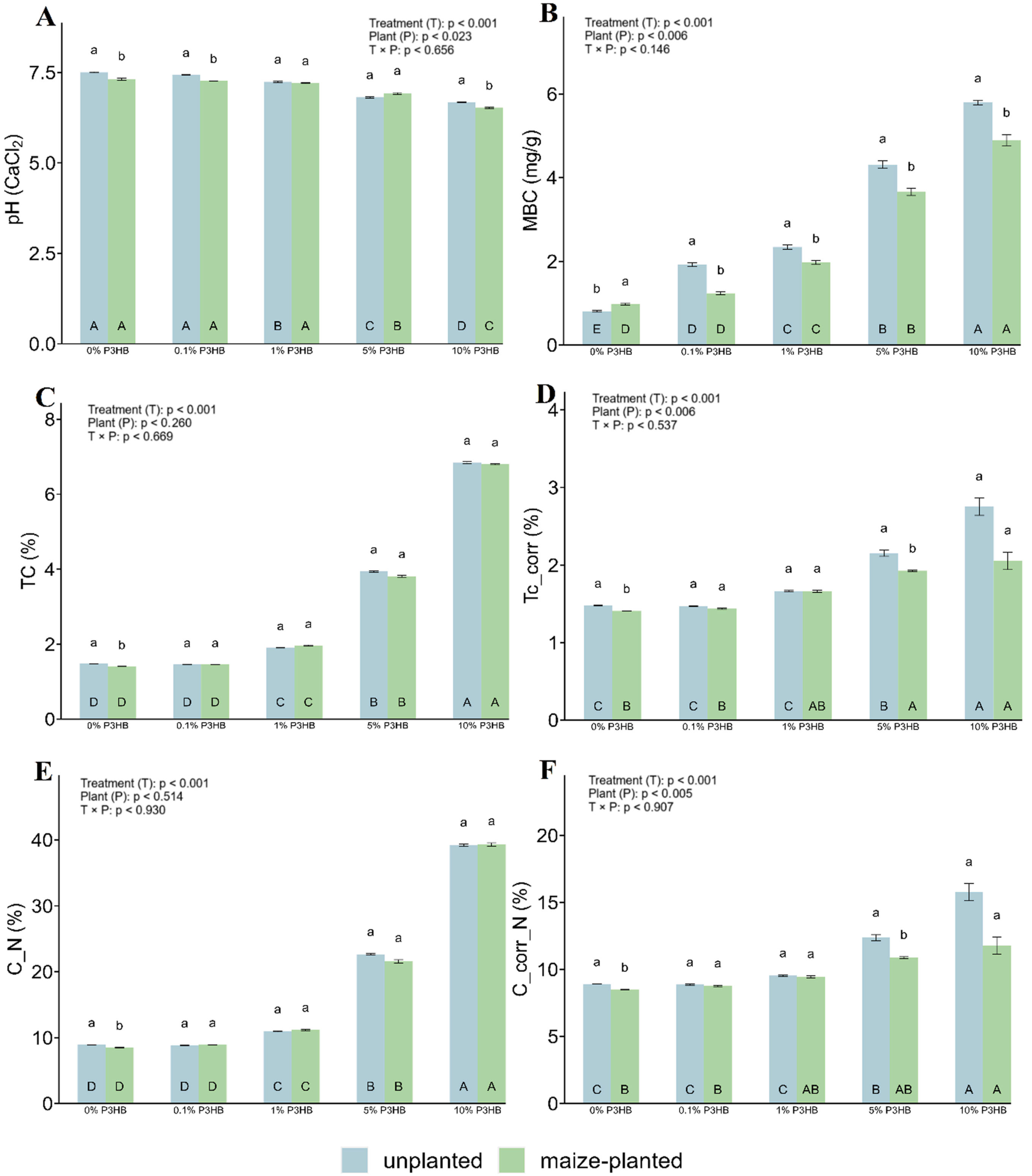

Fig. 1. Effects of poly-3-hydroxybutyrate (P3HB) application on soil physico-chemical properties in unplanted and maize-planted soils. (A) Soil pH, (B) microbial biomass carbon (MBC), (C) total carbon content (TC), and (D) corrected total carbon content (TC_corr, excluding carbon from P3HB residues). Panels (E) and (F) show the carbon-to-nitrogen ratio (C:N) with and without P3HB-derived carbon, respectively. Data are presented as mean values ± standard error of the mean (error bars). Uppercase letters indicate significant differences based on the first independent variable, "Treatment" (P3HB dose), while lowercase letters indicate differences based on the second independent variable, "Plant" (presence or absence of maize), as determined by two-way ANOVA and Tukey's HSD post-hoc test ($p \leq 0.05$).

unplanted soils at each P3HB rate. Fig. 1D reports corrected total carbon (TC_corr; TC without residues of carbon from residual P3HB gradually increased with P3HB amendments. Maize-planted soils had significantly higher TC_corr values than unplanted soils at 5 % and 10 % P3HB. Across all P3HB doses, there was a clear progression in TC_corr, reflecting the influence of P3HB addition. The C:N ratio increased with higher P3HB doses, indicating that P3HB amendments influence the carbon-to-nitrogen balance in the soil. Maize planting contributed to a higher C:N ratio, especially at the highest P3HB doses (Fig. 1E). Similarly to the C:N ratio, the corrected C:N ratio (which excludes P3HB-derived carbon) increased with P3HB amendments, with maize-planted soils generally showing higher values Fig. 1F. The corrected C:N ratio was highest at the 10 % P3HB level, suggesting that increased P3HB additions, along with maize planting, enhance carbon retention relative to nitrogen.

3.2. Soil enzymes

Fig. 2 presents the activities of soil enzymes such as β-glucosidase (GLU), phosphatase (Phos), arylsulfatase (ARS), and urease (Ure), in both unplanted and maize-planted soils amended with different doses of P3HB. GLU activity, generally increased with higher P3HB doses (Fig. 2A). At 5 % and 10 % P3HB, maize-planted soils exhibited significantly higher GLU activity compared to unplanted soils. Among the P3HB doses, significant differences were observed, with the highest enzyme activity at 10 % P3HB. On the contrary, Phos activity (Fig. 2B) remained relatively stable across most P3HB levels, with only minor increases at higher doses. Maize-planted soils tended to show slightly higher phosphatase activity compared to unplanted soils, particularly at 5 % and 10 % P3HB. Differences among P3HB doses were significant, with increased activity at higher P3HB concentrations. Fig. 2C depicts the results of ARS activity that showed a clear dose-dependent increase, particularly notable at 5 % and 10 % P3HB, where maize-planted soils displayed significantly higher activity than unplanted soils. Among P3HB doses, ARS activity was significantly higher at the two highest levels, indicating enhanced sulfur cycling potential with P3HB amendment, especially under planted conditions. Urease activity (Fig. 2D) exhibited the most pronounced increase with P3HB addition, with peak activity observed at 10 % P3HB. Maize-planted soils consistently had higher urease activity than unplanted soils across all P3HB levels. Significant differences were seen across P3HB doses, with a clear upward trend as P3HB concentration increased.

3.3. Soil nutrients

The TN content (Fig. 3A) remained relatively stable across different P3HB doses, with minimal variation between unplanted and maize-planted soils. No substantial changes in TN levels were observed across P3HB treatments, suggesting that P3HB amendments did not significantly impact total nitrogen in the soil. Contrarily, available

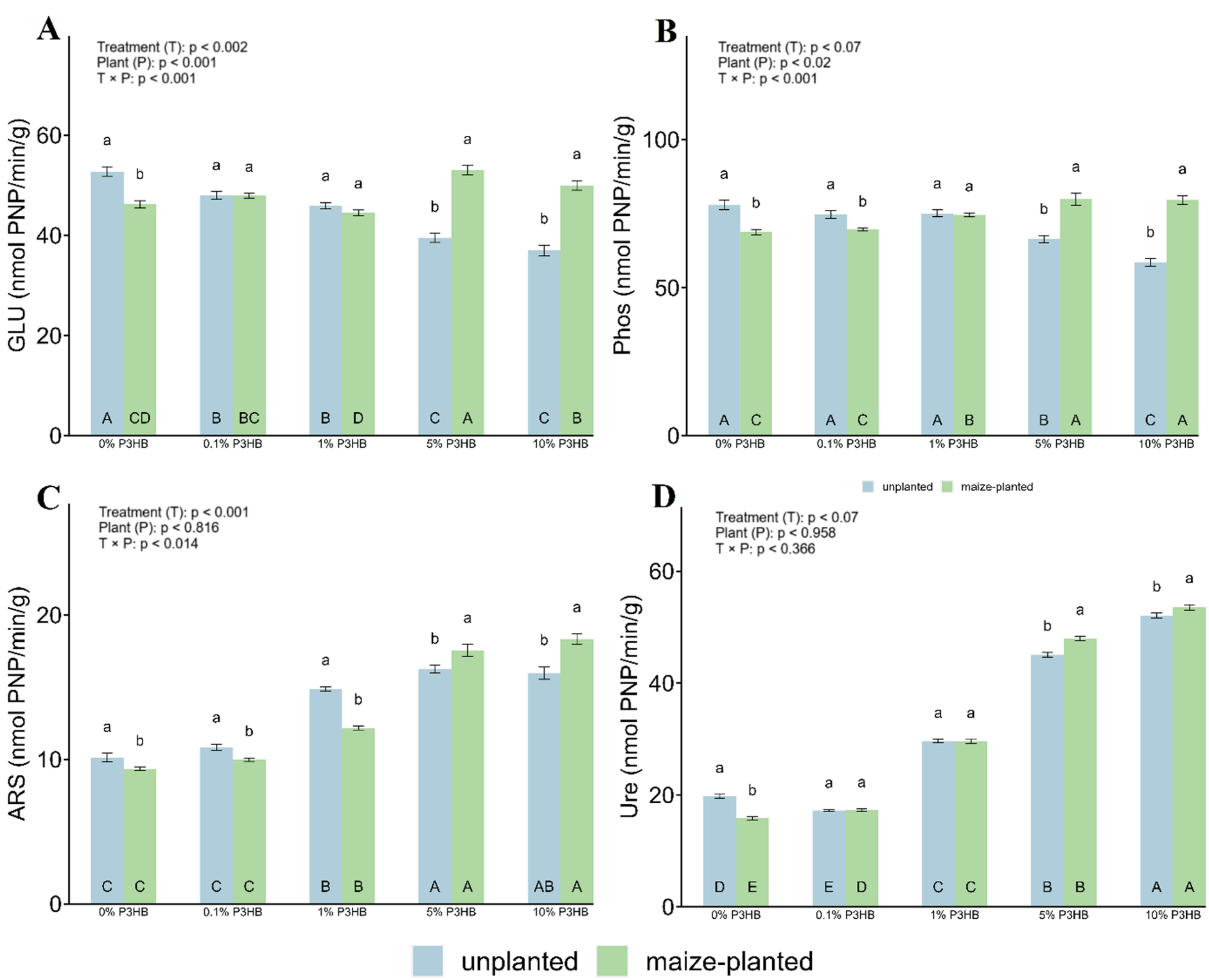

Fig. 2. Enzyme activities in unplanted and maize-planted soils amended with varying doses of poly-3-hydroxybutyrate (P3HB). (A) β-glucosidase (GLU), (B) phosphatase (Phos), (C) arylsulphatase (ARS), and (D) urease (Ure) activities. Data are presented as mean values ± standard error of the mean (error bars). Uppercase letters indicate significant differences based on the first independent variable, "Treatment" (P3HB dose), while lowercase letters indicate differences based on the second independent variable, "Plant" (presence or absence of maize), as determined by two-way ANOVA and Tukey's HSD post-hoc test (p ≤ 0.05).

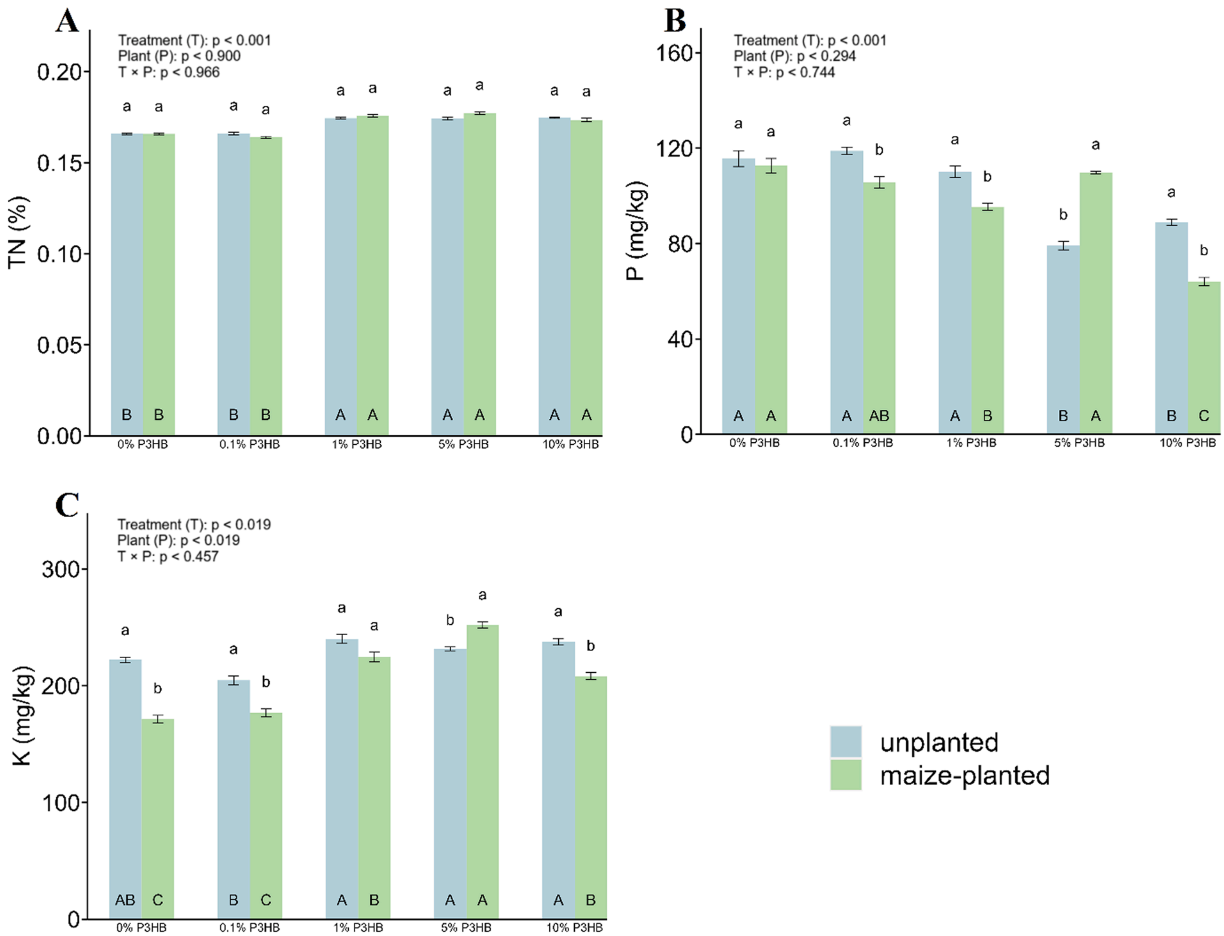

Fig. 3. Soil nutrient content in unplanted and maize-planted soils amended with varying doses of poly-3-hydroxybutyrate (P3HB). Mean values ± standard error of the mean (error bars) are shown for: (A) soil total nitrogen (TN), (B) soil available phosphorus (P), and (C) soil available potassium (K). Uppercase letters indicate significant differences based on the first independent variable, "Treatment" (P3HB dose), while lowercase letters indicate differences based on the second independent variable, "Plant" (presence or absence of maize), as determined by two-way ANOVA and Tukey's HSD post-hoc test ($p \leq 0.05$).

phosphorus (Fig. 3B) showed a decreasing trend with increasing P3HB doses, particularly at 5 % and 10 % P3HB, where phosphorus content was notably lower than at lower P3HB levels. Maize-planted soils had consistently higher phosphorus levels compared to unplanted soils, especially at 0.1 % and 1 % P3HB, indicating that maize planting may help retain phosphorus under certain conditions. The available potassium content increased with higher P3HB doses, especially at 1 %, 5 %, and 10 % P3HB, where maize-planted soils generally showed higher potassium levels than unplanted soils (Fig. 3C). The upward trend in potassium availability with P3HB amendment indicates that P3HB positively influences potassium content, with maize planting providing an additional boost.

3.4. Plant characteristics

Plant nitrogen content (N_biomass) (Fig. 4A) in plant biomass increased significantly with higher P3HB doses, particularly at 5 % and 10 % P3HB, where nitrogen levels were the highest. This trend suggests that higher P3HB amendments enhance nitrogen uptake by plants. Plant phosphorus content (P_biomass) (Fig. 4B) in the plant biomass also increased with P3HB additions, with notable increases from 1 % P3HB onward. Phosphorus levels plateaued at 5 % and 10 % P3HB, indicating that these doses maintain consistent phosphorus availability for plants. Plant potassium (K_biomass) content showed a variable response to P3HB (Fig. 4C), with the highest levels recorded at 1 % and 5 % P3HB. At 10 % P3HB, potassium content significantly decreased, suggesting an optimal range of P3HB amendment for potassium uptake exists, beyond which potassium availability declines. Fig. 4D shows that the highest aboveground biomass was observed in the control and 0.1 % P3HB treatments. Biomass significantly decreased at higher P3HB levels (1–10 %), indicating that high P3HB doses may inhibit biomass accumulation. Plant height (Fig. 4E) followed a similar pattern to aboveground biomass, with the tallest plants observed in the control and 0.1 % P3HB treatments. Plant height significantly decreased at P3HB levels of 1 % or higher, suggesting that while low P3HB levels do not affect plant growth, higher P3HB doses may restrict growth.

Table 2 presents the nutrient uptake calculated as a flux from soil to dry aboveground biomass. The results show a decrease of the flux with increasing dose of P3HB for all the determined nutrients.

3.5. P3HB residues

The residues of P3HB in soil after 90 days of experiment are reported in Table 3. The negative value for unplanted variant amended with 0.1 % P3HB indicates a complete biodegradation and loss of mass in the interval 200–300°C after biodegradation. There is a general trend that planted variants have always higher residual content of P3HB compared to unplanted variant. In addition, the content of residues increases with increasing amendment dose. The negative value in the unplanted 0.1 P3HB variant is caused by the fact, that the P3HB completely degraded, which caused also a small depletion of a labile pool of soil organic matter compared to unamended variant.

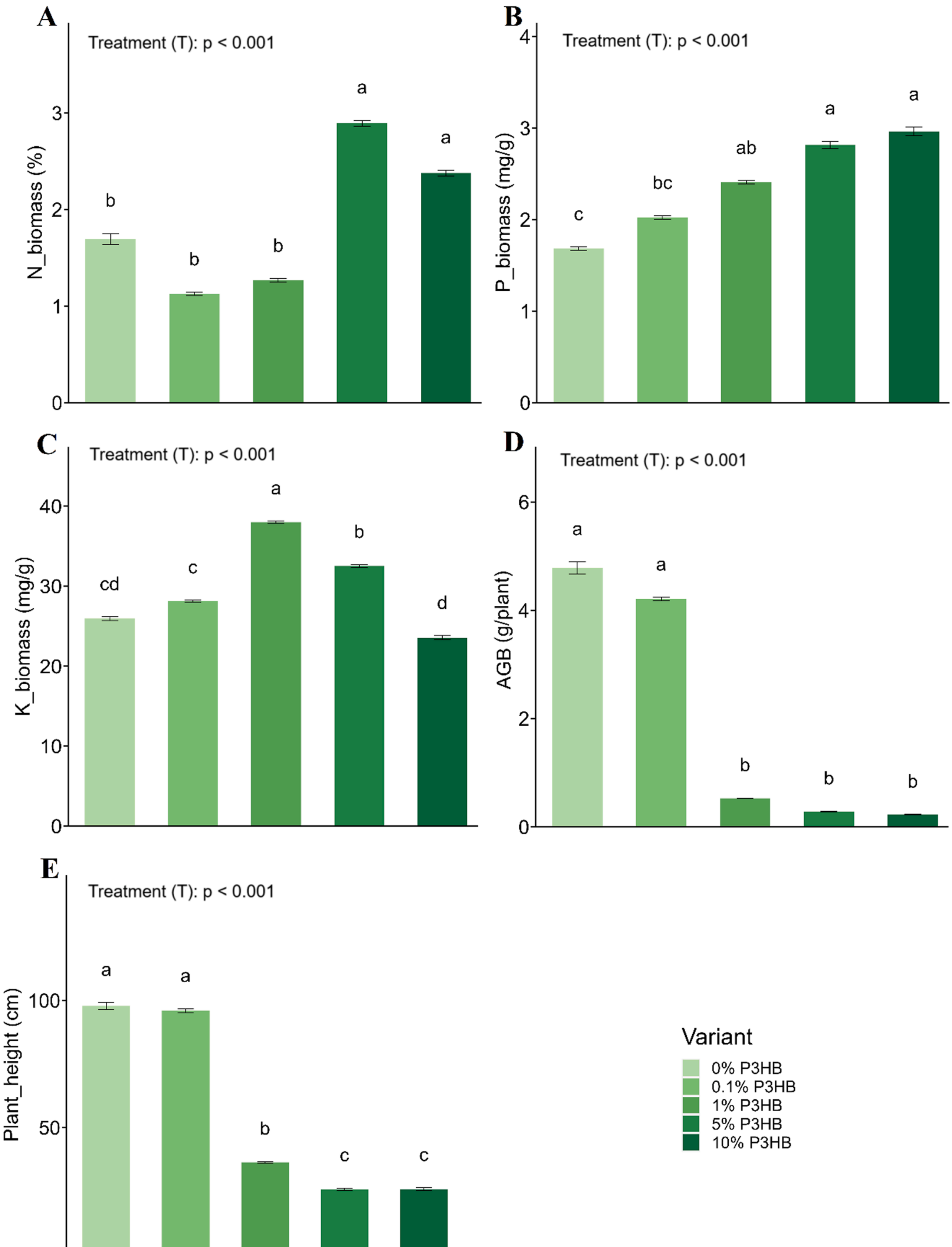

Fig. 4. Plant nutrient content, biomass, and height in maize grown in soils amended with varyingdoses of poly-3-hydroxybutyrate (P3HB). Mean values ± standard error of the mean (error bars) are shown for: (A) plant nitrogen content (N in biomass), (B) plant phosphorus content (P in biomass), (C) plant potassium content (K in biomass), (D) aboveground maize biomass (AGB), and (E) plant height. Letters indicate significant differences based on the independent variable, "Treatment" (P3HB dose), as determined by one-way ANOVA and Tukey's HSD post-hoc test ($p \leq 0.05$).

Table 2
Nutrient uptake calculated as a flux from soil to dry aboveground biomass in variants of maize-planted soil amended with various doses of poly-3-hydroxybutyrate.

variant	N_{uptake} [mg·plant^{-1}]	P_{uptake} [mg·plant^{-1}]	K_{uptake} [mg·plant^{-1}]
P3HB 0 %	7.83 ± 2.2 a	8.05 ± 1.79 a	123.16 ± 23.71 a
P3HB 0.1 %	4.74 ± 0.72 b	8.55 ± 1.26 a	118.59 ± 12.51 a
P3HB 1 %	0.67 ± 0.06c	1.27 ± 0.16c	20.05 ± 1.7 b
P3HB 5 %	0.83 ± 0.2c	0.81 ± 0.22c	9.3 ± 1.92c
P3HB 10 %	0.54 ± 0.1c	0.68 ± 0.17c	5.38 ± 1.01 c

Displayed are mean values (± standard deviation) of nutrient content in biomass mean aboveground biomass yield; lowercase letters show differences among the variants (calculated by one-way ANOVA and Tukey's HSD posthoc according the independent variable, factor "Treatment") at a statistical level of significance $p \leq 0.05$.

Table 3
Residual content of P3HB and content of carbon in total microbial biomass.

P3HB content (%)	**0.1**		**1**	
Variant	planted	unplanted	planted	unplanted
residue (%)	28 ± 3	−14 ± 1*	54 ± 2	44 ± 2
P3HB related carbon (%)	0.04 ± 0.01	−0.01 ± 0.01 *	0.25 ± 0.05	0.18 ± 0.02
P3HB content (%)	**5**		**10**	
Variant	planted	unplanted	planted	unplanted
residue (%)	67 ± 2	63 ± 2	67 ± 2	63 ± 2
P3HB related carbon (%)	0.52 ± 0.08	0.67 ± 0.06	0.52 ± 0.08	0.67 ± 0.06

* it comes from the negative value of the subtraction of mass losses

Table 3 also reports the carbon originating from P3HB (P3HB related carbon). Similarly, as for the residue, in unplanted variants, for the 0.1 % variant the value was negative due to the absence of residual P3HB, in 1 %, 5 % and 10 % P3HB variants can be observed an increasing trend. In planted variants, the trend was also increasing with smaller values compared to unplanted variant.

Fig. 5 illustrate the connection between P3HB-related carbon and P3HB residues with microbial biomass carbon (MBC), respectively. Specifically, Fig. 5A depicts the relationship between P3HB-related carbon and MBC for the planted variant, while Fig. 5B shows the same for the unplanted variant. In both cases, the dependence clearly demonstrates a linear relationship. Fig. 5C and D present the relationship between P3HB residues and MBC for the planted and unplanted variants, respectively. While the relationship appeared linear for the planted variant, the unplanted variant showed that the lowest dosage of P3HB was an outlier and, therefore, was excluded from the linear correlation analysis.

3.6. Relationship between soil and plant characteristics

Figures S1 and S2 report Pearson's correlation of properties in unplanted and planted variants. The correlation coefficients (r) are calculated on the statistical level of significance: $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. The analysis revealed a number of correlations between analyzed parameters discussed in relevant parts of the text.

4. Discussion

4.1. Influence of P3HB biodegradation on soil pH

P3HB, like other polyhydroxyalkanoates, is biodegradable in both soil and compost environments (White et al., 2021) decomposing under anaerobic and aerobic conditions (Altaee et al., 2016). During P3HB depolymerization, 3-hydroxybutyric acid (the protonated form of 3-HB) is released and further oxidized by the dehydrogenase enzyme to acetyl acetate (acetic anhydride) (Kanesawa et al., 1994). Acetoacetate is a reactive compound that hydrolyzes in water to form acetic acid or it is used to react with β-ketothiolase generating acetyl coenzyme A, which is involved in cell regeneration (Kobayashi et al., 2005).

The production of 3-hydroxybutyric acid (pKa ~ 4.41) and potentially of acetic acid (pKa ~ 4.76) (Haynes et al., 2014) may explain the observed decrease in soil pH with increasing P3HB application rates in both unplanted and maize-planted soils (Fig. 1A). Similar acidification effects have been reported for degradation products of polyhydroxyalkanoates in aquatic environments (Sikorska et al., 2015). However, the effects of biodegradable plastics on soil pH are not universally negative. For instance, Dissanayake et al. (2024) conducted a field experiment and found that the degradation of PLA and poly (butylene adipate-co-terephthalate) (PBAT) mulch films did not significantly alter soil pH, with values remaining relatively stable around 6.0 and 5.9. Similarly, Liu et al. (2025) observed that despite the theoretical potential for biodegradable microplastics to lower soil pH, their treatment resulted in an increase in soil pH, likely due to microbial activity that neutralizes acidity. However, our experiment showed a decrease in pH despite enhanced microbial activity, as evidenced by increased enzyme activities such as Phos and ARS (Fig. 2). In addition, the pH decrease was proportional to P3HB content in soil, as reflected by Pearson correlation (Figure S1 and S2), which revealed a strong negative relationship between P3HB residues and pH (Figure S1). This indicates that higher initial P3HB concentrations result in greater residual P3HB in the soil, with microbial degradation continuously producing acids that contribute to pH reduction. Therefore, it can be assumed that the pH decrease may be only temporary and when biodegradation terminates, it can increase as in other studies. In other case, continued degradation would gradually lower pH, especially in soils with limited buffering capacity and acidification could lead to nutrient leaching (e.g., Ca^{2+}, Mg^{2+}), reducing phosphorus availability, and reduction of microbial diversity. Therefore, the long-term effects of biodegradable plastics on soil health, including pH, remain an area requiring further investigation. This aligns to conclusions of Zhang et al. (2022) who recently emphasized the need for more comprehensive studies to understand the long-term impacts of biodegradable film mulching on soil properties, including pH and microbial dynamics.

4.2. Influence of P3HB biodegradation on soil organic matter and P3HB derived carbon

Consistent with findings of Palucha et al., (2024), our study showed that unplanted soils with 0.1 % P3HB achieved complete biodegradation within 90 days as evidenced by thermogravimetric analysis (a negative value in Table 3). This likely reflects the depletion of labile SOM fraction due to a priming effect from enhanced microbial activity. In contrast, planted variants retained higher P3HB residues, highlighting the role of rhizosphere interactions in altering degradation dynamics.

Bioplastic amendments to soil increase organic carbon concentrations (Santini et al., 2022). However, residual P3HB is rarely quantified due to a lack of suitable analytical techniques (Fojt et al., 2020), which can lead to misinterpretation of results. Residual P3HB contributes to total carbon (TC), potentially inflating C:N ratio, especially at high P3HB doses. Corrected TC values reveal more realistic C:N ratios (Fig. 1F) and show that TC and C:N ratios of soil organic matter increased with P3HB doses in both planted and unplanted variants, except at 0.1 % P3HB (Fig. 1D, F). Lower values in planted soils likely reflect lower MBC (and count of microorganisms), which have low C:N (Kästner et al., 2021).

4.3. Microbial incorporation of P3HB carbon

Consistent with previous reports by (Folino et al., 2020; Zhou et al., 2021a), MBC increased proportionally with P3HB content. The

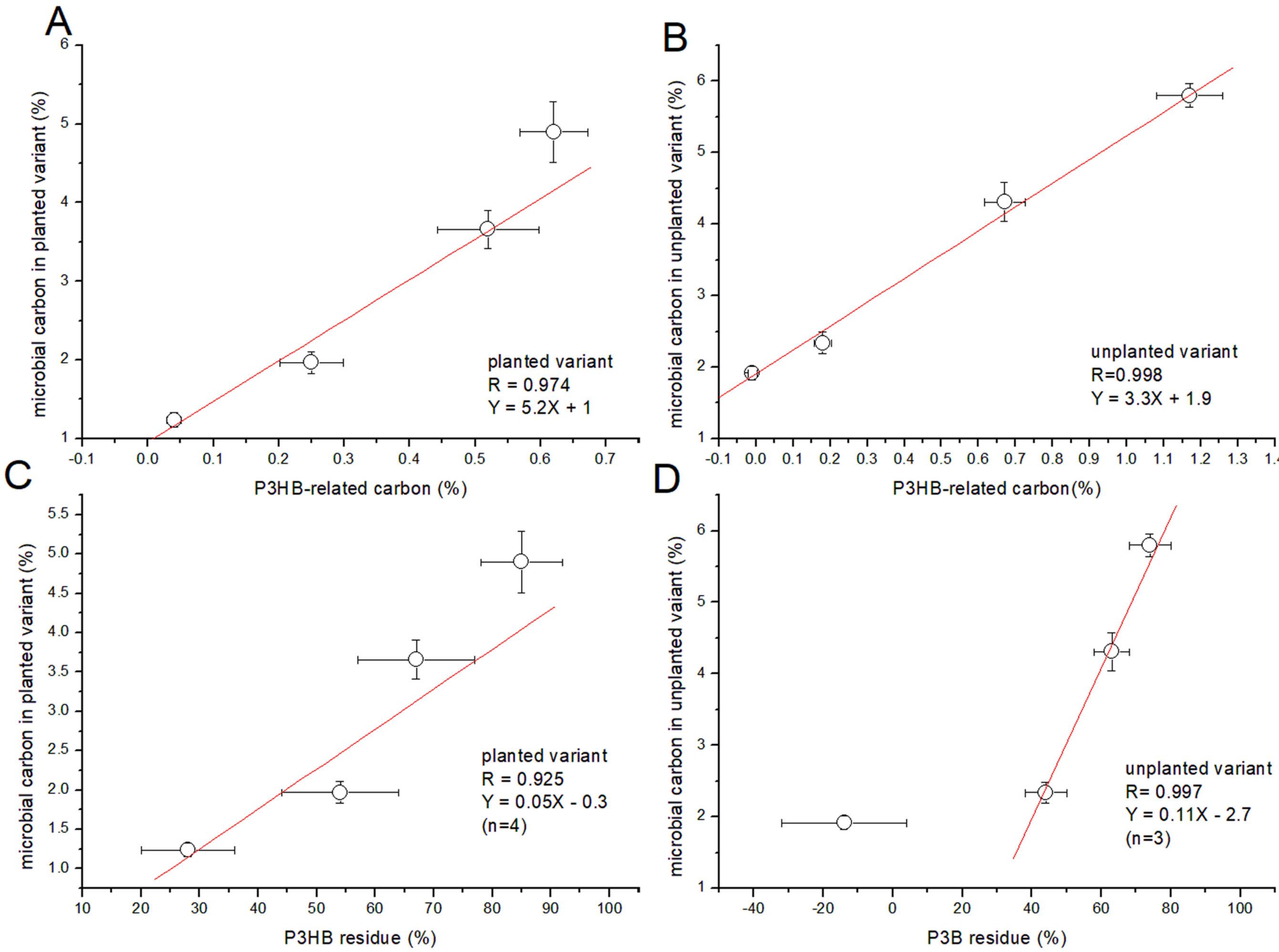

Fig. 5. Relationships between microbial biomass carbon (MBC) and P3HB-related carbon or P3HB residues in planted and unplanted soils. (A) Relation between MBC and P3HB-related carbon in planted soils, (B) Relation between MBC and P3HB-related carbon in unplanted soils, (C) Relation between MBC and P3HB residues in planted soils, and (D) Relation between MBC and P3HB residues in unplanted soils. Data represent relationships in soils amended with varying doses of poly-3-hydroxybutyrate (P3HB).

incorporation of carbon into microbial biomass is reflected in Fig. 5, which shows that the higher P3HB related carbon (from Table 3), the higher was total microbial carbon (MBC). The planted variants showed a steeper MBC increase (slope = 5.2) compared to unplanted soils (slope = 3.3), suggesting greater microbial incorporation of P3HB-derived carbon in rhizospheric environments (Fig. 5). The soil in pot experiment is likely to be dominated by rhizosphere interaction, and therefore, the soils planted variants will lead the primarily plant-microbe interaction (Brown et al., 2023). This may reflect the dominance of rhizosphere interactions, where biota preferentially utilize P3HB degradation products (Musa et al., 2016) or to higher involvement of P3HB in controlling metabolic and physiologic processes of rhizobacteria, e.g. N_2 fixation (Wong and Evans, 1971). Additionally, rhizospheric biota may favor P3HB metabolism over storage due to plant-driven resource competition (Wang and Bakken, 1998).

The rhizosphere microbiome, shaped by plant activities and interactions, acts as a key mediator of nutrient uptake and stress resistance (Zhang et al., 2017). Excess P3HB disturbs this balance by altering carbon availability, nutrient dynamics and microbial competition, potentially enabling pathogen proliferation (Trojan et al., 2024). Recently, it has been demonstrated that microbial inoculation with plant growth-promoting organisms failed to mitigate the negative effects of P3HB, which could only be alleviated with soil amendments like digestate (Brtnicky et al., 2024b; Brtnicky et al., 2024a). The planted soils consistently showed higher P3HB residues, supporting the hypothesis that maize growth was constrained by nutrient competition, leading to lower microbial biomass carbon (MBC) values in planted soils compared to unplanted soils at higher P3HB doses (Fig. 1B).

The relationship between residual P3HB and MBC highlights the broader impacts of P3HB on soil biota. Fig. 5 shows that the relationship is linear in the presence of P3HB in the planted variant. In unplanted soil (Fig. 5D) complete P3HB biodegradation significantly altered microbial community trajectories. Given to the length of the whole experiment, it is likely, that this change can last days to weeks, as suggested by results of Inubushi et al. (2022).

Statistical analyses (Table S1) further confirmed that P3HB treatment levels are the primary drivers of soil physico-chemical properties and plant performance, affecting parameters such as pH, MBC, TC, nitrogen (TN), nutrient ratios, and plant biomass. In contrast, the presence of plants had a smaller but measurable impact, primarily on pH and MBC, underscoring the dominant role of P3HB amendments in modulating soil fertility and plant productivity (discussed in next paragraphs).

4.4. Influence of P3HB biodegradation on soil enzymes

A starvation-survival lifestyle is a typical physiological state of soil microorganisms (Morita, 1997) indicating that only some microbes are active, while others experience arrested activity or enter dormancy, but still retaining the biochemical machinery necessary to rapidly respond to substrate availability (Hobbie and Hobbie, 2013). As P3HB is introduced into the soil, microbial populations produce specific enzymes that catalyze breakdown of P3HB into monomeric units (3-HB causing a pH decrease Fig. 1A). The enzymes include P3HB depolymerase (Yoshie et al., 2002; Zhang et al., 2010) and oligomer hydrolases (Kobayashi et al., 2003), whereas the 3-HB is then cleaved by 3-hydroxybutyrate dehydrogenase (Lu et al., 2014). In biodegradation are involved both fungi (Altaee et al., 2016) and bacteria (Volova et al., 2017), while some of the alter are rhizobacteria with plant-growth-promoting potential (Jeszeova et al., 2018).

Biodegradable bioplastics can modify soil microbial communitieś structure and diversity (Brtnicky et al., 2024b; Lian et al., 2022; Wang et al., 2022b), although some reports found no significant effects (Bandopadhyay et al., 2020). These changes are primarily linked to disruptions in soil nutrient stoichiometry, leading to nutrient imbalance and alleviating carbon limitation (Qi et al., 2020). This leads to a preferential nutrient immobilization in microbial biomass as observed by (Brown et al., 2023) and reflected in Fig. 4. Additionally, a shift in pH is also likely to have significant impact, with a more pronounced effect on bacteria than on fungi (Rousk et al., 2010). These changes are often accompanied by an increase in microbial biomass, as microbes adapt to utilize the substrate more effectively along with alterations in soil enzyme activity (Altaee et al., 2016).

Soil amendment with biodegradable plastics has been associated with an increased β-glucosidase (Santini et al., 2022; Yang et al., 2022) and phosphatase activities (Zhou et al., 2021a). However, both reported trends contrast with our results (Fig. 2), likely due to differences in experimental conditions, such as soil type, microbial community composition, bioplastic types, and environmental factors like temperature and moisture. These results indicate that the influence of bioplastics on soil microbial activity and community structure may be more complex and context-dependent than previously thought.

The divergent patterns observed in β-glucosidase and phosphatase activities may also be attributed to differences between unplanted and maize-planted soils, i.e. (micro)plastisphere versus rhizosphere conditions (Zhou et al., 2021a). In pot experiment, rhizosphere interaction, primarily plant-microbe interaction, are dominant (Brown et al., 2023). Since β-glucosidase activity and phosphatase activities correlated with P3HB dose (Fig. 2A, B), it is likely that the enrichment of P3HB-degrading microbes enhanced soiĺs capacity to depolymerize plant necromass and mobilize phosphorus. Notably, available phosphorus levels in unplanted soil were generally higher than in maize-planted soil, except at 5 % P3HB (Fig. 3B). Although soil phosphorus content declined with increasing P3HB doses, sufficient organic phosphorus reserves and P-solubilizing microbes likely supported enhanced phosphatase activity.

On the contrary, the lower available phosphorus levels in maize-planted soils (0.1 %, 1 %, 10 % P3HB) and the steep decline at higher P3HB levels (5 % and 10 %) suggest competition for phosphorus among plant roots, rhizobiome microbes, and the microplatisphere community leading to reduced phosphatase activity (Fig. 2B). The direct relationship between soil phosphorus and plant biomass (AGB dry) indicates that reduced plant uptake, due to stunted plant growth, did not contribute to phosphorus stabilization in the soil.

In contrast to β-glucosidase and phosphatase, arylsulfatase and urease did not exhibit strong differences between unplanted and maize-planted soils concerning P3HB dose (Fig. 2 C, D). Although sulfate availability was not measured, the increase in arylsulfatase activity between 0 % and 1 % P3HB variants—coinciding with a decline in pH—suggests sulfur mineralization may have contributed to soil acidification. In contrast, the 5 % and 10 % P3HB unplanted variants exhibited stable arylsulfatase and pH levels, potentially indicating sulfur immobilization, as previously reported by Vong et al. (2003). In maize-planted soil, the progressive increase in arylsulfatase from 0 % to 5 % P3HB suggests enhanced microbial sulfur mineralization driven by both microbial and plant sulfur demands under acidifying conditions. A similar stimulation of arylsulfatase activity during biodegradable plastic degradation has been reported by Šárka et al. (2011).

Urease activity showed a direct dependence on P3HB dose in both unplanted and maize-planted soils (Fig. 2D, S6E), in agreement with previous findings for soils amendment with PE microplastics (Yang et al., 2022), biodegradable PLA (Feng et al., 2022; Wang et al., 2022a) and P3HB (Brtnicky et al., 2022). This trend reflects increased nitrogen demand in response to elevated labile carbon availability (Roscoe et al., 2000). Despite a shallower increase in unplanted soils, urease activity remained comparable between unplanted and maize-planted variants across all P3HB doses aligning with the similar total nitrogen (TN) content in both conditions (Fig. 3A).

4.5. Influence of P3HB biodegradation on soil and plant nutrients

4.5.1. Nitrogen uptake by maize in the presence of P3HB

Previous studies on nitrogen dynamics during bioplastic biodegradation have yielded mixed results. Palucha et al. (2024) observed no nitrogen loss in unplanted soils with P3HB over ten month, whereas Feng et al. (2022) reported decreased NH_4^+-N and NO_3−N in soils contaminated with heavy metals after P3HB microplastics addition. Similarly, Liu et al. (2023) observed declining NO_3−N, rising NH_4^+-N and reduced nitrogen content in maize leaves and roots with increasing PLA microplastics doses. Brown et al. (2023) noted NH_4^+-N depletion with increasing poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HBV) concentration, but inconsistent NO_3−N trends. Notably, the plant biomass observed both by Liu et al. (2023) and Brown et al. (2023) decreased with increasing bioplastic dosage, similarly to the trends observed here.

In our study, total nitrogen (TN) initially decreased in soils with 0 % and 0.1 % P3HB, suggesting higher nitrogen flux to plants compared to higher P3HB doses (Fig. 3A). Elevated MBC in high-dose variants likely led to microbial nitrogen immobilization, reducing plant uptake and lowering plant biomass (Fig. 4D). Consequently, nitrogen uptake from soils with low P3HB doses was significantly higher (Table 2). Nitrogen efflux showed an exponential decrease with increasing P3HB suggesting a threshold between 0.1 % and 1 % P3HB for safe application, contrasting with (Brown et al., 2023), who proposed a P3HBV below 0.01 %. Our results indicate that 0.1 % P3HB had an insignificant effect on maize biomass, whereas 1 % P3HB significantly reduced growth. However, as presented by (Palucha et al., 2024), the application of P3HB (or fast biodegrading polymers in general) in the form of mulch could lead to local hotspots, with concentrations exceeding 1 %, which makes those considerations situation-dependent.

From the plant-uptake perspective, nitrogen content in maize biomass increased at 5 % and 10 % P3HB doses, likely due to nutrient accumulation in stressed tissues (Fig. 4A). This contrasts with Liu et al. (2023), who attributed nitrogen reduction to the PLA biodegradation products supporting microbial proliferation and fixation, reducing the uptake of essential nutrients. Brown et al. (2023) reported increased foliar C:N ratio in P3HBV-treated soils, with stress markers indicating inefficient energy production and/or nutrient imbalance and potential P3HBV-induced root hypoxia. Given P3HBVś chemical similarity to P3HB, differences in nitrogen dynamics may stem from variations in metabolic products (i.e 3-hydroxypropanoic acid and 3-hydroxypentanoic acid for P3HBV), warranting further investigation.

4.5.2. Phosphorus uptake by maize in the presence of P3HB

Phosphorus is critical for plant metabolism, root development (Panchal et al., 2021) and stress tolerance (Shrivastav et al., 2020). Similar to nitrogen, phosphorus efflux from soil decreased with increasing P3HB doses (Table 2). However, unlike nitrogen, soil phosphorus availability did not increase with P3HB amendment in either unplanted or maize-planted soils (Fig. 3B). A significant phosphorus decline was observed only at 5 % and 10 % P3HB in unplanted variants.

Therefore, the phosphorus flux to plants does not correlate with soil phosphorus content, contrary to what was observed for nitrogen (Fig. 4B, S2).

Phosphorus solubilization depends on optimal pH (6−7) and microbial activity (Spohn and Kuzyakov, 2013). Additionally, dilution of initial phosphorus levels by added P3HB mass may have contributed to lower phosphorus availability. These results align with Sigmon et al. (2023), who found that PHA coatings on phosphorus fertilizers enhanced phosphorus uptake and reduced run-off. Similarly, Zhang et al. (2023) reported reduced available phosphorus in paddy soils amended with poly(butylene adipate terephthalate) (ranging from 0.1 % to 1.0 %) due to phosphatase inhibition. Our results suggest that the reduced run-off may be associated with the immobilization of phosphorus by enhanced microbial activity and MBC.

Phosphorus plays a critical role in stress tolerance such as drought, high salinity, or extreme temperatures (Raghothama, 1999; Vance et al., 2003). Consistent with this, maize phosphorus content increased with increasing P3HB amendment (Fig. 3B), likely due to enhanced plant demand under stress. However, Pearson correlation did not confirm an increase in phosphatase activity (0.32; Figure S2). Instead, pH decrease due to P3HB degradation (-0.67***; Figure S2) likely contributed to increased phosphorus solubilization (Zhang et al., 2021) and uptake. These findings suggest that phosphorus flux in P3HB-amended soils is governed by pH changes, microbial activity, and plant demand, and likely also moisture shortage, which can occur in P3HB hotspots due to elevated desiccation (Fojt et al., 2022).

4.5.3. Potassium uptake by maize in the presence of P3HB

Potassium plays a central role in plant stress response (Cakmak, 2005), water transport and nutrients uptake (Panchal et al., 2021; Shrivastav et al., 2020). In unplanted soils, available potassium remined stable across P3HB treatments (Fig. 4C). In maize-planted variants, potassium uptake decreased with increasing P3HB doses (Table 2). While 0 % and 0.1 % P3HB showed similar values, amending with 1 % P3HB significantly increased available potassium accompanied by higher plant biomass potassium content, suggesting that higher microbial biomass likely enhanced potassium retention in the soil. However, at 5 % P3HB, soil potassium peaked, coinciding with a sharp decrease in plant potassium content, which dropped further declined at the 10 % P3HB dose.

P3HB effects on potassium are poorly documented, but susceptibility to desiccation stress (Xu et al., 2021) suggest that high P3HB doses (5 %, and 10 %) may have induced drought stress reducing potassium uptake. P3HB also significantly reduced maize aboveground biomass at higher doses (Fig. 4) mirroring trends observed in grasses (*Thinopyrum junceum)* (Menicagli et al., 2023), soybeans (*Glycine max)* (Li et al., 2021) and lettuce (*Lactuca sativa*) (Brtnicky et al., 2024b). These studies attributed the biomass loss to plant-microbe competition for nutrients.

As observed for phosphorus, drought stress may have exacerbated potassium deficiency at high P3HB doses. Potassium deficiency is known to impair water status and stem diameter dynamics making plants more vulnerable to drought (Kanai et al., 2011), whereas adequate potassium availability improves water-stressed plant growth (Bahrami-Rad and Hajiboland, 2017). Maize biomass (AGB_dry) and plant height declined at higher P3HB doses, potentially due to inhibitory effects of P3HB degradation intermediates, similar to PLA-induced stress reported by (Liu et al., 2023).

Last, fluctuating potassium content in plant biomass (Fig. 3C) suggests nutrient prioritization under stress (Richardson et al., 2011; Vance et al., 2003). Even at low doses (0.01 % and 0.1 %), both conventional and biodegradable microplastics can alter root exudation and rhizosphere function by inducing nutrient deficiencies (Zhou et al., 2021a). Potassium deficiency triggers sugar-based root exudation (glucose, ribitol, fructose, maltose), while phosphorus and micronutrient deficiencies (e.g., zinc, iron) promote organic acid exudation (Panchal et al., 2021). P3HB-indiced pH reduction likely favored uptake of nutrients other than potassium, as evidenced by correlations between enzyme activities and biomass nitrogen/phosphorus but not potassium (Figure S1B). Nevertheless, further research is needed to elucidate how biodegradable plastics reshape rhizobiome composition and dynamics.

5. Conclusions

Although pot experiments have certain limitations compared to field study (which are rare in the literature), the results of this study underscore the dual impacts of excessive P3HB on soil health and crop performance, highlighting a critical trade-off between soil quality improvements and crop productivity. While P3HB amendment enhanced soil microbial activity, organic carbon content, and enzyme activities, higher application rates (≥1 %) triggered nutrient competition, limiting the availability of essential nutrients for plants and ultimately reducing maize biomass.

Therefore, although P3HB biodegradation can improve soil quality, its agricultural application requires careful management to avoid detrimental effects on crop productivity. As biodegradable plastics represent a valuable strategy to reduce persistent plastics fragments in soil, a balanced approach that considers both soil health benefits and crop needs is crucial. In particular, future research should focus on i) optimizing P3HB application rates to maximize soil quality improvements without compromising crop growth, ii) understand the long-term impacts of P3HB on soil-plant-microbe interactions and, iii) developing strategies to improve carbon use efficiency of biodegradable plastics and to amplify their positive impacts on soil̓s chemical, physical and microbiological health.

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CRediT authorship contribution statement

Skarpa Petr: Writing – review & editing, Methodology, Data curation. **Mustafa Adnan:** Writing – review & editing, Validation, Supervision. **Siddiqui Manzer H.:** Writing – review & editing, Supervision, Funding acquisition. **Hammerschmiedt Tereza:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis. **Naveed Muhammad:** Writing – review & editing, Validation, Supervision. **Kintl Antonin:** Validation, Resources, Methodology, Funding acquisition, Formal analysis. **Baltazar Tivadar:** Visualization, Software, Resources, Data curation. **Holatko Jiri:** Writing – review & editing, Writing – original draft, Validation, Supervision, Funding acquisition, Conceptualization. **Brtnicky Martin:** Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Kucerik Jiri:** Writing – original draft, Validation, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manzer H. Siddiqui reports financial support was provided by King Saud University. Jiri Holatko reports was provided by Ministry of Agriculture of the Czech Republic. Antonin Kintl reports financial support was provided by Ministry of Agriculture of the Czech Republic. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118131.

Data availability

Data will be made available on request.

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ORIGINAL PAPER

Effect of stabilized organic amendments on biodegradability of poly-3-hydroxybutyrate, soil biological properties, and plant biomass

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Abstract

Poly-3-hydroxybutyrate (P3HB) is a biodegradable polymer with a potential extensive use in agriculture. However, while P3HB biodegradation boosts microbial enzyme activity, it significantly reduces plant biomass due to nutrient competition. In this study, we test the hypothesis that these detrimental effects can be mitigated though the co-application of nutrient-rich organic amendments, such as compost and digestate. A pot experiment with lettuce (*Lactuca sativa*), grown in soil amended with P3HB lone or combined with either compost or digestate. Six variants were tested: Control, Compost, Compost + P3HB, Digestate, Digestate + P3HB, and P3HB alone. We evaluated degradation of the P3HB polymer, biological soil properties, and both the dry and fresh biomass of the lettuce. We observed that adding P3HB alone enhanced dehydrogenase and urease activities, as well as all types of respiration, except for L-arginine-induced respiration. However, it strongly and negatively affected the biomass of lettuce (both aboveground and root). The strong adverse effects of P3HB on plant growth were also observed when compost was co-applied, although this combination enhanced all enzyme activities except for suppressed *β*-glucosidase. Conversely, co-applying digestate with P3HB alleviated the negative effect of P3HB on both the dry and fresh biomass together lettuce. Additionally, this combination increased the activity of several enzymes (dehydrogenase, arylsulfatase, *N*-acetyl-*β*-*D*-glucosaminidase, urease), and enhanced all types of respiration, except for *L*-arginine-induced respiration. The use of biodegradable plastics in agriculture is on rise, but it may be compromised, because their biodegradation my negatively impact plant growth. The results showed that co-application of digestate is an effective solution to alleviate these effects, while co-application of compost failed. Generally, organic amendments seem to be an option to alleviate the negative effects of bioplastics biodegradation, and offers options how to handle the treatment of waste bioplastics or their residues, but further investigation is needed to understand the underlaying mechanisms involved.

Graphical Abstract

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Keywords Biodegradable plastics · Stabilized organic matter · Soil degradation · Microbial activity · Soil enzymes · Soil respiration

Abbreviations

3-HB	3-Hydroxybutyric acid
AGB dry	Dry aboveground lettuce biomass
AGB fresh	Fresh aboveground lettuce biomass
ANOVA	Analysis of variance
ARS	Arylsulfatase
BR	Basal respiration
C_{input}	Amount of carbon added to soil with amendments
Co	Compost
DHA	Dehydrogenase activity
DEHP	Di(2-ethylhexyl)phthalate
Di	Digestate
GLU	*β*-Glucosidase
EOM	External organic matter
IR	Substrate induced respirations *D*-glucose (Glc-IR), D-trehalose (Tre-IR), *N*-acetyl-*β*-*D*-glucosamine (NAG-IR), *L*-alanine (Ala-IR), *L*-lysine (Lys-IR) and *L*-arginine (Arg-IR)
MFD	Microbial functional diversity
NAG	*N*-Acetyl-*β*-*D*-glucosaminidase
N_{input}	Amount of nitrogen added to soil with amendments
p	*p*-Value
PHA	Polyhydroxyalkanoates
P3HB	Poly-3-hydroxybutyrate
Phos	Phosphatase
r	Pearson's correlation coefficient
SOM	Soil organic matter
Ure	Urease
WHC	Water holding capacity

Introduction

Increasing production of biodegradable polymers provides a broad range of materials, including the biopolyesters belonging to the group of polyhydroxyalkanoates (PHAs) (Sudesh et al. 2000). PHAs are produced by numerous prokaryotic species during fermentation of carbohydrates, usually under growth-limited conditions such as nutrient deficiencies (Grousseau et al. 2013; Lee 1996). Their physical and chemical properties make them suitable substitutes for conventional nonbiodegradable plastics (Bonartseva et al. 2003). PHAs are biodegradable, biocompatible, and thermoprocessible (Medeiros Garcia Alcantara et al. 2020). In agriculture, they can be used for mulching (as cover films), in pots and containers (as bands for sowing), and for the controlled release of agricultural chemicals (Touchaleaume et al. 2016; Vroman and Tighzert 2009).

Nowadays, among the most notable PHAs belongs poly-3-hydroxybutyrate (P3HB), which serves as a bacterial intracellular carbon (C) and energy storage polymer. The complete degradability of PHAs, especially P3HB, is a characteristic that has made them widely used and environmentally friendly polymers (Vroman and Tighzert 2009; Luckachan and Pillai 2011).

P3HB has been found to biodegrade under both anaerobic and aerobic conditions (Nishida and Tokiwa 1993; Sharifzadeh et al. 2009) in soil, compost, and water bodies (Deroiné et al. 2015; Savenkova et al. 2000; Altaee et al. 2016; Bonartsev et al. 2018). Enzyme activities of P3HB-depolymerases and hydrolases have been identified in several microbial taxa (Kadouri et al. 2003; Shah et al. 2007; Panayotidou et al. 2014; Roohi and Kuddus 2018). The soil´s capacity for the P3HB degradation is largely determined by the biota structure, which includes macrodegraders, mostly earthworms (Sanchez-Hernandez et al. 2020) and microbial community (Abou-Zeid et al. 2004; Rychter et al.2006).

Indeed, the microbial community structure is often referred as the key factor in the rapid and efficient biodegradation of P3HB materials in soil (Guo et al. 2010; Vogel et al. 2021). However, the activity of P3HB-degrading microbes is influenced by balances and nutritional state at various levels of soil metabolism. The rate and the soils ‘ microbiome capacity to degrade P3HB depend on the availability of limiting nutrients (Nishide et al. 1999; Sang et al. 2004; Muneer et al. 2020; Zhou et al. 2021a)). Nitrogen, a crucial nutrient in the decomposition medium (Corrêa et al. 2008), must be supplied during soil P3HB degradation, either via co-application with compost (Eya et al. 1997) or through pre-degradation of P3HB in composted organic matter (Eya et al. 1997; Rosa et al. 2023).

Due to the origin of P3HB, the non-toxicity of its particles and degradation products is assumed. Nevertheless, this assumption is increasingly being challenged by available studies. Only a few studies have assessed the effect of PHA-amended soil on plant growth, and they reported negative effects (Zhou et al. 2021a; Dahal et al. 2020). For example, in our recent work (Brtnicky et al. 2022) we tested the impact of P3HB addition on biological properties of a wide range of nutrient-restricted soil substrates and lettuce growth. It was observed that P3HB addition increased dehydrogenase and urease activities, and basal and substrate-induced respiration in nutrient-restricted soils. Furthermore, it appeared that in those soils, P3HB can temporarily replace the SOM as a C source for microbial communities. Nevertheless, the addition of P3HB to all soils, tested in cited work, showed a negative impact of biodegradation on lettuce growth.

On the contrary, the potential negative effects of P3HB degradation products on terrestrial or freshwater organisms are well recognized (González-Pleiter et al. 2019). In addition, the P3HB degradation product such as 3-hydroxybutyric acid (3-HB) has been reported to play a significant signaling role in the global regulation processes of eukaryotic cells (Puchalska and Crawford 2017). In a previous study, the amendment of exogenous 3-HB to flax plants (*Linum usitatissimum* L.) resulted in changes to the DNA de-/methylation pattern, which potentially impacts the expression of genes involved in the phenylpropanoid pathway (Mierziak et al. 2020). Changes in the biosynthesis of phenylpropanoids, which help to inactivate reactive oxygen species, may alter plant responses to abiotic stresses conditions (Sharma et al. 2019). Malik et al. (Malik et al. 2015) further reported that in transgenic plant *Camelina sativa,* where the P3HB constituted up to 15% of mature seed weight, there were negative effects on germination, emergence, and survival of seedlings. Therefore, there are compelling reasons to evaluate and investigate the factors, strategies, and management practices for controlled biodegradation of P3HB-based plastics in the soil.

In addition, soil hydrophysical properties are among the traits known to be affected by poly-3-hydroxybutyrate and other plastic residues. Wan et al. (Wan et al. 2019) referred that the addition of plastic to soil creates channels and alters water movement, leading to negative impacts on water evaporation. De Souza Machado, on the contrary, observed an increased water holding capacity for polyester, and no effects for polyacrylic and polyethylene fragments on soil properties in a five-week experiment (Souza Machado et al. 2018). The authors hypothesized about possible connection with the impact on soil aggregation. Fojt et al., (Fojt et al. 2022) demonstrated that poly-3-hydroxybutyrate particles changed the soil organic matter by altering its supramolecular structure. Such behavior of plastics in soil affects water holding capacity (WHC) and dynamics, which could impact plant-available water, plant growth and fitness. However, applying organic amendment, such as compost, to P3HB-enriched soil may aid in restoring WHC values as well as providing nitrogen supplementation to preserve degradation capability and activity (Rosa et al. 2023; Suzuki et al. 2007).

In this work, we continue our previous research (Brtnicky et al. 2022) that enabled understanding the issues related to the impact of P3HB biodegradation on soil under various content of soil organic matter and soil texture. The main issue appeared the competition between soil microorganisms and plants for nutrients, which cannot easily be solved by inoculation of soil by plant growth-promoting rhizobacteria and N_2-fixing microorganisms, as shown recently (Brtnicky et al. 2024). Apparently, this issue can be solved by a direct supply of nutrients, the most feasible is the addition of either a fertilizer or a nutrient-rich amendment. However, NPK fertilizers, particularly those with higher nitrogen content in the form of ammonium or urea, tend to decrease soil pH over time (Hao et al. 2020). Although the P3HB-related acidification is not the primary factor inhibiting plant growth (released 3-hydroxybutyric acid has pKa of 4.41) (Bruss et al. 2008), addition of NPK fertilizers may worsen this issue. On the contrary, the application of stabilized amendments such as digestate or compost generally increases soil pH, and possibly counterbalance the effect of 3-HB and the nutrient shortage during biodegradation. In addition, use of such amendments is more sustainable and less demanding to soil heath compared to application of synthetic NPK fertilizers (Panuccio et al. 2019).

To the best of our knowledge, this approach has not been tested up to now. However, understanding the effects of organic amendments sources on the degradability of P3HB and the overall soil microbial and physico-chemical properties is vital for strategies for bioplastics waste disposal and also application of fast biodegradable plastics in agriculture, which is on rise (Santagata et al. 2017) in applications such as mulching, fertilizer coating and delivery of active compounds (Touchaleaume et al. 2016; Vroman and Tighzert 2009).

Therefore, this study aimed to evaluate the effect of solely applied poly-3-hydroxybutyrate (P3HB) and P3HB co-applied with stabilized organic matter (compost or digestate) on the biological properties of soil and the biomass of lettuce (*Lactuca sativa*).

It was hypothesized that:

1. P3HB is utilized as a readily catabolized energy and carbon source in soil, resulting in enhanced degradation and respiration, but it reduces the intrinsic utilization and transformation of soil organic carbon.
2. Plant aboveground and root biomass is decreased in P3HB-amended variants compared to unamended ones, due to competition from metabolic active and abundant soil microorganisms with plants for nutrients and due to the negative impact of P3HB on soil hydrophysical properties.
3. Organic amendments provide more nutrients, enhance the transformation and mineralization activities of soil organisms, and increase respiration rate. They can

improve plant biomass yield compared to both control and P3HB-affected plants.

4. The organic amendment, by improving soil properties and nutrient status, will accelerate the degradation of biodegradable P3HB.

Materials and methods

Experimental design and treatment description

The study was carried od as a short-termed pot experiment under controlled conditions in growth chamber. The soil used for preparation of the experimental substrate was an arable Haplic Luvisol (WRB soil classification), silty clay loam (USDA Textural Triangle) (WRB Soil Classification 2021). It was a topsoil (0–15 cm), collected at field near Troubsko town, Czech Republic (49°10′28″ N 16°29′32″ E), with the following properties: pH ($CaCl_2$) 7.3; total C 14.0, total N 1.60, S 0.145, P 0.097, K 0.231, Ca 3.26, Mg 0.236 g kg^{-1}. Before use, a sieving (through a mesh) to size ≤ 2 mm was done (Hammerschmiedt et al. 2022). This sieved soil was mixed with a fine quartz sand (0.1–1.0 mm; ≥ 95% SiO_2) in weight ratio 1:1 to gain an experimental substrate. One kg of experimental substrate was mixed with additives (Table 1) and put into 1-L plastic pots (height 13 cm, top diameter 11 cm, bottom diameter 9 cm) (Przygocka-Cyna et al. 2018). 3 replicates (pots) were prepared for each treatment. Commercial CMC compost (Fertia s.r.o., Czech Republic; in fresh matter C 127.9, N 11.8 g kg^{-1}, C:N 10.8) and digestate (in fresh matter C 14,2, N 1.59 g kg^{-1}, C:N 8.9) obtained from agricultural biogas plant were used as additives.

The pots were seeded with lettuce (*Lactuca sativa* L. var. *capitata* L.) cv. Brilliant, 3 sprouted lettuce seeds per each pot and cultivated in growth chamber under controlled conditions (as follow): photoperiod 12 h (Cervera-Mata et al. 2018), light intensity 20 000 lx (Bankole et al. 2018; Zhang et al. 2018), relative air humidity 70% (Chrysargyris et al. 2018), night/day temperature 18/22 °C; soil moisture was ~ 60% of water holding capacity. The one most robust plant was left in each pot after 10-day-growth of seedlings. A randomized placement of pots in the growth chamber was used and once per week, the pots were variably rotated (Iocoli et al. 2019). After 8 weeks from sowing, the plants were harvested by cutting the shoots at ground level (Trinchera et al. 1041). The roots were released from soil, cleaned gently and washed with water. The fresh lettuce aboveground and roots biomass was weighed on the analytical scales. The dry biomass was determined after drying the shoots and roots at 60 °C to a constant weight and weighting on the analytical scales again.

Poly-3-hydroxybutyrate (P3HB) used in this work was obtained from TianAn Biologic Materials Co., Ltd. (Ningbo City, China), grade ENMAT Y3000, powder with particle size < 63 µm. The particles were of spherical shapes with density of approximately 1.20 g·cm^{-3} (Höhnemann and Windschiegl 2023). The crystallinity of the polymer was 49% (Procházková et al. 2024). Further specifications of the used P3HB is reported in Fojt et al. 2022 and Y3000P 2023 (Fojt et al. 2022; Y3000P 2023).

Soil analysis

A mixed soil sample was taken from experimental substrate of each pot after harvesting the lettuce. Samples were sieved on mesh to size ≤ 2 mm and used for determination of soil properties, with 3–6 analytical replication (according to measured property) per each sample replication. The soil pH of air-dried samples was determined (ISO_10390 2005). The samples used for determination of dehydrogenase activity (DHA) (Ranamukhaarachchi 2009), soil basal respiration (BR) and substrate induced respirations (IR) were stored at 4 °C. Soil respiration analyses were detected using MicroResp® device (The James Hutton Institute, Scotland), which performance is regularly verified according to the instructions of the provider. The tests were carried out according to Campbell et al. (2003), with following induction substrates–*D*-glucose (Glc-IR), *N*-acetyl-*β*-*D*-glucosamine (NAG-IR), *D*-trehalose (Tre-IR), *L*-lysine (Lys-IR), *L*-alanine (Ala-IR), and *L*-arginine (Arg-IR). The values of induced respiration were used to calculate the

Table 1 Experimental treatments (and amendments doses added to 1 L of soil)

Treatment	P3HB [g]	Digestate [mL]	Compost [g]	C_{input} [g·kg^{-1}]	N_{input} [g·kg^{-1}]
Control	–	–	–		
Digestate	–	32	–	0.45	0.05
Compost	–	–	32	4.09	0.38
P3HB	5	–	–	2.33	–
Digestate + P3HB	5	32	–	2.78	0.05
Compost + P3HB	5	–	32	6.42	0.38

C_{input}, N_{input}–amount of carbon and nitrogen, added to soil with amendments (P3HB, compost or digestate) microbial functional diversity (MFD) according to Iovieno et al. (2021) as a Shannon's index according to equation:

$$MFD = -\Sigma pi \ln pi \tag{1}$$

pi = the activity on a particular substrate with respect to the sum of activities on all substrates.

Enzymatic activities were measured in the freeze-dried samples: β-glucosidase (GLU), N-acetyl-β-D-glucosaminidase (NAG), phosphatase (Phos), arylsulfatase (ARS), urease (Ure) (ISO 2013 0:2018). The p-nitrophenole (PNP)-derivatives of the specific soil substrates were used for Vis spectrophotometric measurement (Infinite M Nano, Tecan Trading AG, Switzerland) at $\lambda = 405$ nm (β-glucosidase, N-acetyl-β-D-glucosaminidase, phosphatase, arylsulfatase). Urease activity was determined as an amount of ammonium produced from the substrate urea, detected Vis spectrophotometrically by the reagent cyanurate ($\lambda = 650$ nm). Using enzyme activities, nutrient acquisition ratios were calculated, based on the formulae presented in Cui et al. (2022):

$$\text{acquisition ratio} = \ln(DHA + GLU)/\ln(DHA + GLU + NAG + Ure) \tag{2}$$

$$N \text{ acquisition ratio} = \ln(NAG + Ure)/\ln(NAG + Ure + Phos) \tag{3}$$

When demand of soil microbiome for carbon increases and more carbon utilizing enzymes are secreted to obtain C, this results to higher C acquisition ratio. Similarly, when soil microorganisms are deficient for nitrogen, they produce additional enzymes catalyzing N decomposition, resulting in higher N acquisition ratio.

The theory of enzymatic stoichiometry (Moorhead et al. 2016) was used to compute the vector length and angle in order to estimate the microbial resource limitation. Microbial C limitation aggravates with the increase in the vector length. While the vector angle of < 45° indicates microbial N limitation, the vector angle > 45° indicates microbial P limitation. The following formulae based on (Moorhead et al. 2016) were used, ARCTG2 refers to arcus tangens:

$$\text{Vector length} = \sqrt{\left(\frac{\ln(DHA + GLU)}{\ln(Phos)}\right)^2 + \left(\frac{\ln(DHA + GLU)}{\ln(NAG + Ure)}\right)^2} \tag{4}$$

$$Vectorangle(rad) = ARCTG2\frac{\ln(DHA + GLU)}{\ln(Phos)};\frac{\ln(DHA + GLU)}{\ln(NAG + Ure)} \tag{5}$$

The quality control (QC) and quality assurance (QA) of the used devices and obtained data was performed as follows: QC: Analytical equipment used for enzymatic assays, respirometry, balances and thermogravimetry are regularly calibrated and verified, to ensure accuracy in measurements. In some cases (enzymes, respirometry), we also incorporate positive and negative controls in experiments, i.e. positive controls ensure the assay is capable of producing a positive result when expected, and negative controls confirm the absence of contamination and non-specific signals. All measurements are done at least in triplicate or more to assess the reproducibility of the results. In addition, standard curves for quantification in enzymatic assays and respiration are used to ensure linearity of the detection range and to validate the assay's sensitivity and specificity. QA: We keep all detailed documentation of all protocols of standardization and procedures used in the experiments to unsure that the procedures can be repeated under the same conditions and produce similar results. All personnel involved in the experiments are properly trained and competent in the specific techniques and equipment used. We implement a process for checking the raw data for errors or inconsistencies before analysis, i.e. cross-checking data entries, verifying calculation methods, or using software for data integrity checks.

Thermogravimetric analysis (TG)

The thermogravimetric analysis was conducted in order to analyze residual plastic in soils after termination of the experiment. The instrument was calibrated for temperature using Curie point of alumel, nickel and iron; mass loss was controlled by calcium oxalate degradation. These values were verified regularly each month to assure the accurate performance of the device. The analysis was conducted using thermogravimeter Q550 TA Instruments (Delaware, USA). The homogenized soils were placed to the pre-weighed alumina pans and heated from laboratory temperature up to 650 °C, with heating rate 5 °C min^{-1} under the dynamic air atmosphere enriched by water vapors to 43% relative humidity, flow rate 90 ml min^{-1}. The TG records were elaborated using TRIOS from TA Instruments. Measurements of each variant were done in triplicate, the obtained mass losses were averaged. To obtain the residual P3HB, the TG data were treated according to the procedure reported recently by Palucha et al. 2024 (Palucha et al. 2024). Briefly, as the pure P3HB thermally degrades in the interval from 200 to 300 °C, mass loss obtained in this temperature area of the control sample was subtracted from the respective sample containing P3HB and adjusted for dry mass by considering the moisture contents (100% minus mass loss in the interval 25–200 °C). Results of repeated measurements were averaged and standard deviation was calculated.

Statistical analyses

The Software R, version 3.6.1. (R_Core_Team. R 2020) was used for the following data processing and statistical analyses to detect statistically significant difference among factor level means through methods of one-way analysis of variance (ANOVA) type I (sequential), using sum of squares at 5% significance level (Zar 1984), Tukey's HSD (honestly significant difference) test and "treatment contrast" to calculate factor level means for each treatment. The linear dependence between all soil properties was determined by Pearson's correlation analysis to reveal the mutual relationships between individual enzymes, IR and yield of plants. The interpretation of Pearson's correlation coefficient (r) was as follows: $0.0 < r < 0.3$ (negligible correlation), $0.3 < r < 0.5$ (low correlation), $0.5 < r < 0.7$ (moderate correlation) and $0.7 < r < 0.9$ (high correlation), $0.9 < r < 1.0$ (very high correlation) (Hinkle et al. 2003). After all statistical analyses the assumptions of selected models was also checked at significance level of 0.05. For testing the normality, it was used Kolmogorov–Smirnov test and for testing the homoscedasticity, it was used Bartlett's test of homogeneity of variances. Besides, the model checking was also performed using different diagnostic plots.

Results and discussion

Effect of P3HB on plant aboveground, root biomass, and enzyme activity

The application of P3HB significantly reduced the abovegound fresh and dry biomass (AGB fresh, AGB dry) of lettuce when applied alone or in combination with compost (Co) (Fig. 1a, b). Compost alone did not enhance AGB fresh and AGB dry compared to the control, while digestate (Di) notably improved lettuce biomass, both alone and when combined with P3HB. Moreover, digestate co-applied with P3HB mitigated the decline in AGB fresh and AGB dry caused by P3HB alone, even surpassing the biomass in the Co-only variant (Fig. 1a). However, both P3HB and P3HB + compost treatments negativelly impacted root biomass (fresh and dry) compared to the control. In contrast, Di co-applied with P3HB improved root dry biomass, though it remained lower then in the digestate, compost, and control treatments (Fig. 1d). Fresh root biomass varied significantly across treatments (Fig. 1c), with the highets fresh biomass observed in the digstate-amended soils.

Soil degradation activities, as indicated by dehydrogenase (DHA), increased across all amended variants compared to the control, particularly in the Di + P3HB variant, highlighting enhanced carbon mineralization (Fig. 2a). Conversely, *β*-glucosidase (GLU) activity, another carbon mineralizing enzyme, was suppressed by P3HB with significant enhancement only observed with digestate addition (Fig. 2f). P3HB presence in the soil reduced carbon transformation efficiency, as indicated by lower C acquisition ratios (Table 2). Higher vector length values (Table 2) in P3HB-unamended variants indicated greater C limitation.

Arylsulfatase (ARS) activity increased across all treatments, particularly in the Co + P3HB variant (Fig. 2c). Enzymes involved in nitrogen mineralization, such as *N*-acetyl-*β*-*D*-glucosaminidase (NAG) and urease (Ure), were mainly elevated by P3HB, whether applied alone or with digestate or compost (Fig. 2b and d). The co-application of P3HB with digestate further boosted NAG activity, with the highest increase seen with digestate alone, while the combination P3HB and compost led to the highest Ure activity (Fig. 2d). Phosphatase (Phos) activity was enhanced under combined treatments, most notably in the Co + P3HB variant (Fig. 2e).

Statistical analysis (i.e. the correlation matrix in Figure S1) supported above-statements and revealed P3HB

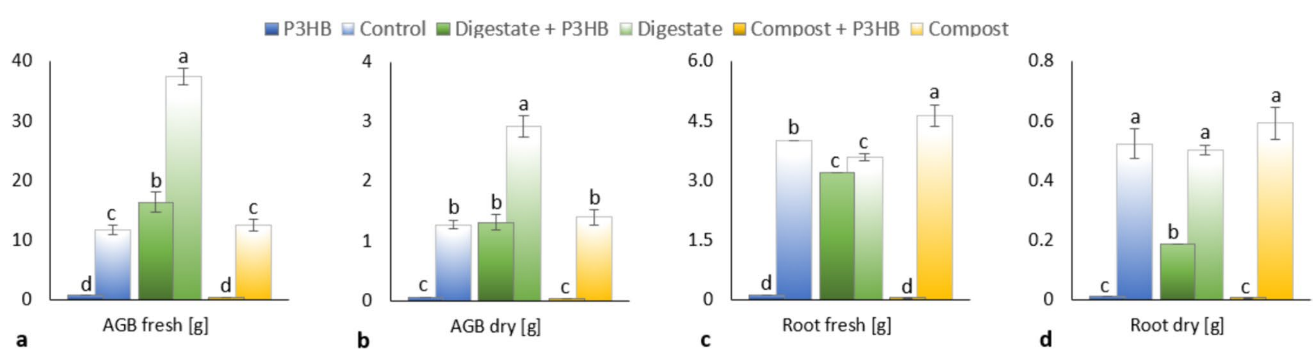

Fig. 1 Plant biomass of treatments with P3HB, compost, digestate and combination Plant fresh **(a)** and dry aboveground biomass **(b),** fresh **(c)** and dry root biomass **(d)**, lowercase letters indicate the differences between variants on the statistical significance level $p \leq 0.05$

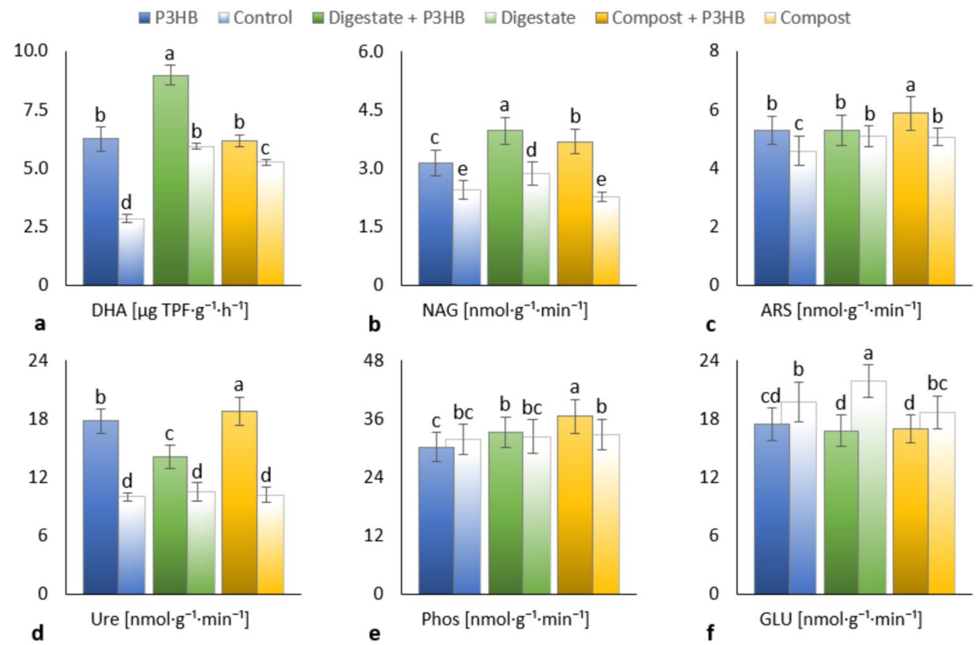

Fig. 2 Enzyme activities of the soil amended with P3HB, compost, digestate, and combination Dehydrogenase activity **(a)**, *N*-acetyl-*β*-*D*-glucosaminidase **(b),** arylsulfatase **(c)**, urease **(d),** phosphatase **(e)** and *β*-glucosidase **(f)** activities. Lowercase letters indicate the differences between variants on the statistical significance level $p \leq 0.05$

Table 2 The carbon and nitrogen acquisition ratios and C, N, P limitation indicators in the soil amended with P3HB, compost, digestate, and combination

variant	C acquisition ratio	N acquisition ratio	C, N, P limitation Vector	Angle	° (angle degrees)
P3HB	0.83	0.77	1.39	0.84	48.24
Control	0.88	0.67	1.53	0.94	53.86
Digestate + P3HB	0.86	0.74	1.46	0.88	50.44
Digestate	0.89	0.68	1.60	0.93	53.23
Compost + P3HB	0.82	0.76	1.33	0.86	49.15
Compost	0.88	0.66	1.55	0.94	54.12

treatment strongly correlates with reduced aboveground biomass (AGB) and root biomass, as indicated by significant negative correlations with glucose ($r = -0.48$ for AGB fresh) and glucosamine ($r = -0.62$ for root dry). Conversely, dehydrogenase activity (DHA) showed a positive correlation with glucose and trehalose respiration ($r = 0.64$ and 0.67, respectively), highlighting P3HB's impact on carbon cycling enzymes. Next, microbial activity, particularly *β*-glucosidase (GLU) and arylsulfatase (ARS), appeared to be inversely related to plant biomass under P3HB treatment, with significant negative correlations with root dry weight ($r = -0.53$ and -0.22, respectively).

Effect of P3HB on soil respiration

The introduction of stabilized organic matter (compost or digestate) did not significantly enhance basal respiration (BR) or substrate induced respiration (IR) compared to the control. In fact, digestate reduced all respiration indicators, and compost specfically decreased Glc-, Tre-, NAG, and Arg-IR relative to the control (Fig. 3). On the contrary, P3HB alone stimulated all forms of respiraton except Tre-IR and Arg-IR (Fig. 3c, g). The co-application of P3HB with compost generally reduced (BR, Tre-IR, Lys-IR) or mainained (Glc-, NAG-, Ala-IR) respiration levels compared to P3HB alone, though Glc- and Ala-IR in the P3HB + Co were higher than in the control. When P3HB was combined with digestate, it significantly enhanced all types of IR except for Arg-IR, which was higher in

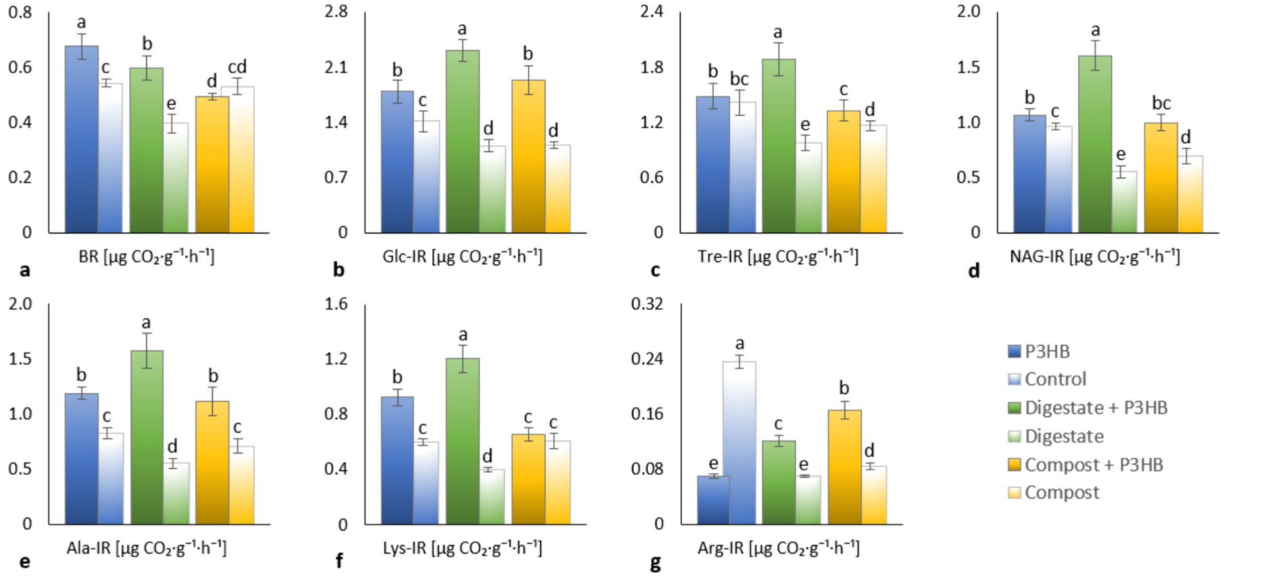

Fig. 3 Respiration of the soil amended with P3HB, compost, digestate and combination Basal respiration **(a)** and substrate-induced respirations—Glc-IR **(b)**, Tre-IR **(c)**, NAG-IR **(d)**, Ala-IR **(e)**, Lys-IR **(f)**, Arg-IR **(g)**. Lowercase letters indicate the differences between variants on the statistical significance level $p \leq 0.05$

Table 3 The microbial functional diversity (MFD) in the soil amended with P3HB, compost, digestate, and combination

Variant	MFD
P3HB	1.623
Control	1.672
Digestate + P3HB	1.637
Digestate	1.603
Compost + P3HB	1.632
Compost	1.636

the P3HB + Di variant than in P3HB alone, but still lower then in the control (Fig. 3e). Interestingly, BR was more elevated in the P3HB treatment than in the P3HB + Di combination (Fig. 3a).

The interactions among various respiration types were evident, as demonstrated by strong positive correlation between Glc-IR and other types including Tre-IR, NAG-IR, Ala-IR, and Lys-IR, with correlaton coeffiients of 0.8, 0.88, 0.92, 0.83, respectively (Figure S1). Additionally, Glc-IR correlated with NAG ($r = 0.82$), indicating interderdence among these respiration activities. Depite these correlations, the independence of these respiration types were reflected in the microbial functional diversity (MFD parameter), which showed any amendment reduced the functional diversity of the soil microbiome. Suprisingly, the sole addition of digestate had the most pronounced negative effect on diversity, while all three PHB-based treatments displayed comparable reductions in MFD (Table 3).

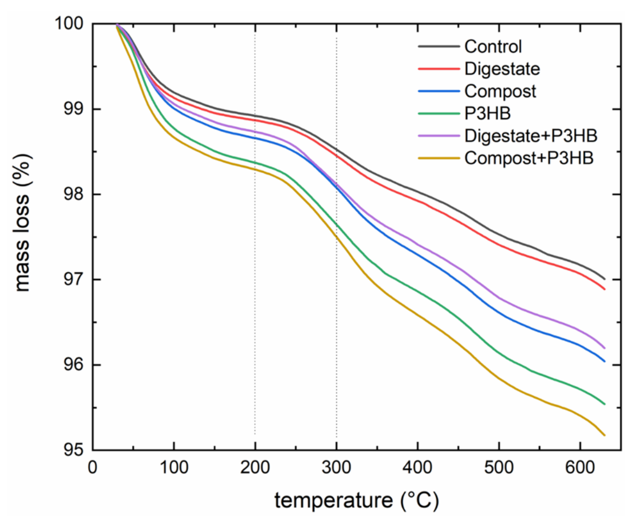

Fig. 4 Thermogravimetry records of soil amended with P3HB, compost, digestate and combination, measured from laboratory temperature to 650 °C with indicated temperature range from 200 to 300 °C for determination of residual P3HB

Thermogravimetry results

Figure 4 presents exemplary thermogravimetric (TG) results, showing mass loss percentages as the temperature increases in an oxidative atmosphere, ranging from laboratory temperature to 650 °C. According to Palucha et al. 2027 (see Sect. "Thermogravimetric analysis (TG)"), mass loss, particularly between 200 and 300 °C, corresponds to the degradation of P3HB together and the labile components of SOM, as indicated by dotted lines in the Fig. 4 (Palucha et al. 2024). Among all soils tested, the control exhibited the least mass lost, with progressively greater losses observed in soil amended with compost and digestate, and the most significant loss in soils treated with P3HB.

Residual P3HB content was quantified post-heating, revealing $0.37 \pm 0.05\%$ (from the original 1%) residual P3HB in the control soil, reduced to 0.22 ± 0.05 and $0.23 \pm 0.04\%$ in soils amended with digestate and compost, respectively.

Discussion

Effect of P3HB on plant aboveground and root biomass, and enzyme activity.

Effect on plant aboveground and root biomass

The effect of P3HB (applied solely or with compost) on both abovegound and root fresh and dry biomass (AGB fresh, AGB dry, Root fresh, Root dry) of lettuce was strongly negative. Brtnicky et al. 2022 (Brtnicky et al. 2022) reported a similar deleterious impact of P3HB on plant growth in both soil and soil-sand substrates (Brtnicky et al. 2022). In general, the adverse impact of P3HB on plant growth can be explained as the enhanced turnover of SOM and competition of highly active microbial biomass in degradation hotspots (Zhou et al. 2021b) and plants for nutrients (Zhou et al. 2021a). This competition subsequently enhances nutrient transformation and reduces their sequestration for plant nutrition, particularly phosphorus and nitrogen. It has been reported that a similar increase in the input of easily available carbon (C) into the rhizosphere (Kuzyakov and Xu 2013; Nguyen 2003) may escalate microbiome-plant competition due to higher microbial abundance, activity, and growth in the rhizosphere (Blagodatskaya et al. 2010). Consequently, this drains off the remaining nutrients available to plants due to higher microbial uptake and immobilization (Zak et al. 2000). This explanation is supported by the negative correlation between AGB fresh, AGB dry, Root fresh, Root dry and indicators of nitrogen mineralization such as Ure (r values were −0.61, −0.73, −0.94, −0.94) and Ala-IR (r values were −0.5, −0.56, −0.43, −0.71). The P3HB variant showed a higher nitrogen value acquisition ratio in comparison to the control (Table 2), which corroborates our hypothesis #2.

Morevover, the amendment of compost did not effectively nourish the lettuce, as its application increased signficantly only the root fresh biomass compared to the control. On the contrary, solely applied digestate had a positive impact on both dry and fresh AGB (Fig. 1a, b), albeit it showed a slightly negative effect on root fresh biomass (Fig. 1d). When applied with P3HB, digestate alleviated the deleterious impact of bioplastics on all determinants of lettuce biomass yield and even incerased the AGB fresh biomass more than the compost amendment. The properties of digestate, such as higher water content and readily available ammonium nitrogen, were presumably responsible for mitigation the P3HB-mediated repression of plant growth, despite potentially lower total nitrogen input (Table 1). These findings partially (in the case of digestate) confirmed our hypothesis #3.

As mentioned above, the increased water evaporation/drain-off, leading to decreased water content should be considered in P3HB-amended soil. The P3HB is a water insoluble material with a reported contact angle between 70° (Bonartsev et al. 2013) and approximately 81° (Pompe et al. 2007), thus, it slightly increases water repellency. Niu et al. (Niu et al. 2016) noted that the evaporation coefficient increased with higher doses of residual plastic film in soil, suggesting a decreased water holding capacity. Wan et al. (Wan et al. 2019) reported an increased rate of soil water evaporation with a higher amount of plastics in soil, and Fojt et al. (Fojt et al. 2022) observed a decrease in evaporation enthalpy after the adition of P3HB particles to soil organic matter. On the contrary, digestate amendment to soil could have a positive, evaporation-mitigating effect on soil and enhance the availability of water for plants, as referred by some authors (Beck-Broichsitter et al. 2020; Nabel et al. 2017). This feature, along with the reported beneficial effect of digestate on plant growth and the abundance of plant-promoting rhizobacteria (PGPR) in soil (Siebielec et al. 2018), might explain why the Di + P3HB variant did not exert any significant negative effects on plant biomass.

Effect on soil enzymes

DHA was enhanced by all tested soil amendments, but more significantly with P3HB and digestate, and to a lesser extent with compost. The addition of P3HB induced soil degradation as this compound was primarily utilized as a carbon and energy source by microbes (Bonartseva et al. 2003; Rizzarelli et al. 2004), and the results correspond to previous findings (Brtnicky et al. 2022). An even higher induction of P3HB-derived carbon mineralization was expected under conditions of higher nitrogen access (and also other nutrients) in the variants co-amended with P3HB and stabilized organic matter. However, only the P3HB + Di amendment lead to higher DHA compared to the sole P3HB addition, as shown in Fig. 2a. There was a markable tendency towards higher carbon limitation in P3HB-unamended variants compared to those supplied with bioplastics (Table 2). This difference in nutrient availability and the interplay between bioplastics, P3HB-degrading microbes, and the plant rhizobiome presumably mediated the enhanced DHA activity in a similar manner as observed recently (Bai et al. 2020) when di(2-ethylhexyl) phthalate (DEHP) promoted increased biodegradation under soil bioaugmentation with PGPR and changes in dominant genera (*Allorhizobium, Neorhizobium, Pararhizobium, Rhizobium, Caulobacteraceae*) in the consortium. Similar changes in the microbiome composition can be inferred from the differences in the microbial functional diversity (MFD), which significantly varied mainly between the control and all P3HB-based variants (Table 3). This presumption was supported by the observations that several types of respiration showed positive correlations with DHA: Glc-IR ($r = 0.64$), Ala-IR ($r = 0.67$), Lys-IR ($r = 0.67$, Figure S1).

In contrast to DHA, other enzymes (especially GLU) were more likely involved in the transformation of intrinsic SOM (or external organic matter, EOM, of compost and digestate amendments). GLU was lower in the presence of P3HB than under amendment of digestate or compost, or without any amendment (Fig. 2f), suggesting that P3HB may mitigate the mineralization of cellulose and its derivatives in soil. This observation aligns with findings from Brtnicky et al. 2022 (Brtnicky et al. 2022) and with reports of P3HB being used as a single carbon and energy source (Bonartseva et al. 2003; Rizzarelli et al. 2004), which is preferred over organic C from either SOM and extraneous organic matter (EOM) of stabilized organic amendments (compost and digestate). This assumption could be related to suprisingly unchanged values of of the C acquisition ratio (Table 2) in P3HB-supplied variants. In these variants, values of the ratio were relatively decreased due to the mitigated carbon uptake from intrinsic SOM, as most of the utilized carbon was derived from P3HB. This finding verified our hypothesis #1. Significant GLU enhancement was achieved only with the addition of digestate, which possibly also provided higher inoculation with cellulolytic microbes.

Although ARS activity was induced by all tested amendments (most significantly by P3HB + Co), it only showed a weak correlation with other enzymes, except for Ure (where $r = 0.59$, Figure S1). Poly-3-hydoxybutyre did not significantly stimulate ARS compared to the solely applied digestate or compost. Therefore, no P3HB specific impact was ascribed to the results, aligning with the findings of Brtnicky et al. 2022 (Brtnicky et al. 2022). The highest ARS value (Fig. 2c) was presumably due to the highest available sulphur content in the compost, as shown by Prasad et al. 2022 (Prasad et al. 2012), and the increased microbial biomass due to the supply of P3HB.

NAG, an indicator or fungal biomass degradation and turnover, was found to be significantly increased in all P3HB variant (compared to the control), similarly as referred in Brtnicky et al. 2022 (Brtnicky et al. 2022). However, the combinations of P3HB + Co and P3HB + Di were even more beneficial for NAG activity (Fig. 2b). It was observed P3HB in the soil contributed to the multiplication of saprophytic fungi, similarly as reported e.g. by the study (Janczak et al. 2020), and these fungi exhibit the ability to catabolize P3HB (Altaee et al. 2016; Sang et al. 2002). The significant increase in NAG, whether due to P3HB-, digestate- (alone or with P3HB) or compost, also indicated an enhancement of nutrient (including nitrogen) mineralization due to increased depolymerization of P3HB. The mutual relationship between nutrient uptake due to partial organic matter degradation and related fungal biomass turnover was documented by a positive correlation between DHA and NAG ($r = 0.67$, Figure S1).

Furthermore, another nitrogen mineralizing enzyme, urease (Ure), was induced by the addition of P3HB to soil, whether applied alone or in combination with digestate or compost (Fig. 2d). The highest Ure value was found in soil amended with P3HB and compost, presumably due to the highest nitrogen dose provided by compost (Table 1). These results, similar those Brtnicky et al. 2022 (Brtnicky et al. 2022), aligned with reports of higher early losses of available nitrogen in soil amended with either compost or digestate (Nicholson et al. 2017). However, the enhancement of Ure activity was significantly related to the simultaneous application of P3HB, as neither the Ure values nor the nitrogen acquisition ratios of the digestate, compost, and control differed markedly. The related enhanced N acquisition ratios in P3HB-amended variants were found to be significantly higher than in the unamended variants (Table 2). The relationship between Ure and catabolism in SOM (and carbon mineralization) was documented by a positive correlation of Ure with either GLU ($r = 0.63$) or Al-IR ($r = 0.54$) (Figure S1). The results of NAG and Ure (and partially also ARS) determination corroborated Hypothesis #3: i.e. organic amendments provided more nutrients, enhanced their transformation mineralization activities.

Phosphatase activity, similar to the results in Brtnicky et al. 2022 (Brtnicky et al. 2022) and to ARS, was mostly insignificantly affected either by P3HB or stabilized organic matter, with the only significant effect detected in the P3HB + Co variant (Fig. 2e). This was due to the presumed highest phosphorus availability in this blend, an assumption that aligns with reported general levels of phosphorus in various stabilized organic matters (Prasad et al. 2012; Manasa et al. 2020). Values in Table 2 showed that biodegradable plastic played role in higher nitrogen acquisition, whereas P3HB-unamendment variant appeared much less limited by nitrogen than by phosphorus. The Angle values (Table 2) indicated that increased degradation of organic matter in all amended variants was likely the reason for phosphorus limitation (compared to the weakest limitation in the control), but this limitation was least severe (lowest value) in the P3HB + Co soil. Again, presumably the highest phosphorus content led to significantly higher Phos activity.

Effect of P3HB on soil respiration

Among all three amended materials, only the P3HB amendment lead to an increase in respiration potential compared to the control (as previously was reported in Brtnicky et al. 2022 (Brtnicky et al. 2022)), indicated by stimulated respiration of all types except for Tre-IR and Arg-IR (Fig. 3c, g). These results might also be presumably due to a P3HB-derived amplification of aerobic degraders, which corroborates our hypothesis #1. On the contrary, the digestate amendment decreased all respiration indicators, presumably due to the second lowest input of organic C in external organic matter (Table 1), which likely also had higher recalcitrance than the more labile P3HB-associated organic carbon. Moreover, the minimal respiration activity of the digestate and compost variants, coupled with the lowest carbon mineralization, was caused by carbon limitation (with the lowest values being 1.6, 1.55, respectively, as shown in Table 2). Compost added to the soil decreased Glc-, Tre-, NAG, and Arg-IR compared to the control (Fig. 3).

Co-amendment of P3HB with stabilized organic matter (compost or digestate) further altered the respiration potencial (and likely the abundance of aerobes) in comparison to the single P3HB amendment (Fig. 3). When P3HB was co-applied with compost, it decreased BR, Tre-IR, Lys-IR, despite the presumed availability of nutrients (nitrogen, sulphur, phosphorus) inferred from the values of enzyme activities (NAG, Ure, ARS, Phos). Some adversely acting factors could be assumed, such as low plant root biomass and assumed lower water content (compared to the digestate-treated variant), which slightly negatively effected microbial respiration in the soil compared to the impact of sole P3HB. The important role of soil mositure in the degradation of plastic residues in soil has already been described (King et al. 2015). The study by Almethyeab et al. (Almethyeb et al. 2013) showed how amplified PGPR and root symbionts significantly affected plant growth and nutrient uptake, and in return, increased soil respiration. In contrats, the P3HB co-applied with digestate was presumed to cause the most significant amplification of aerobic soil degraders, as indicated by the enhancement of all IR types except for Arg-IR. The most significant decline in Arg-IR (in comparison to the control) across all IRs in P3HB, P3HB + Co, and P3HB + Di variants (Fig. 3g) possibly indicated that the functional fraction of the soil microbiome, capable of utilizing L-arginine (Arg) utilization, was also affected by plant–microbe competition for amino acids, as described in study (Owen and Jones 2001). This competition was expected to be highest particularly in P3HB and P3HB + Co variants. Therefore, the results of respiration determination led to the partial rejection of Hypothesis #3, that posited that organic amendment(s) should enhance both nutrient transformation (mineralization) activities and respiration rate, as the respiration types in sole compost- or digestate-amended variants were mitigated. The variability in the response of each respiration type to respective amendments was best documented by the parameter MFD (microbial functional diversity, Table 3), which as it showed that each amendment reduced functional diversity of the soil in comparison to the control, leading to a less diverse microbiome in P3HB-treated soil and the lowest diversity in the digestate variant.

Effect of organic amendments on P3HB degradation

As demonstarted by this and previous works (e.g. (Zhou et al. 2021a; Brtnicky et al. 2022)) the biodegradation of polyalkanoates is connected with a demand for nutrients, in particular nitrogen and phosphorus. In addition, the presence of plastic residue accelerates moisture evaporation (Fojt et al. 2022) thereby stressing plants due to a lack of moisture. The TG results obtained here suggest that biodegradation was incomplete when the experiment ended, with both P3HB degrading organisms and residues of P3HB still present in the soil. In addition, it was obseved that the rate of P3HB degradation was higher in soils amended with compost and digestate, while it was lower in control soil. This supports the Hypothesis 4 that the amendment accelerates the degradation of biodegradable P3HB. However, the degree of degradation in compost and digestate correlate neither with the enzyme content nor with the AGB results. In particular, compost apeared to be a good substrate for P3HB biodegradation, but, at the same time, did not support the plant growth. On the contrary, digestate supports both biodegradation and plant growth. Considering that Ure levels were higher in compost and Phos were comparable in both variants, we can only speculate that this may be related to the mechanisms of nutrient fixation in both substrates and their differing impacts on soil physical properteis (porosity, density).

To the best of our knowledge, no study has compared those amendments in terms of their influence on the rate of biodegradation. On the contrary, the effectiveness of digestate comparing to compost in promoting plant growth and enhancing soil biochemical processes has been studied and the authors obtained various results (Aguilar-Benítez et al. 2020; Tambone and Adani 2017). We conclude that the specific effect would depend on feedstock for compost and digestate production, technoogy used for their production, soil type and many other factors, which were not included in this study. Thus, understanding this problem requires futher research involving different composts and digestates obtained from various feedstocks, various soil types mainly in terms of soil texture and soil organic matter contest, various temperatures and moisture levels. Nevertheless, in light of the results obtained in this study, the application of an amendment providing nutrinets and water and suporting the soil aeration seem to be necessary to mitigate the negative effects of bioplastic biodegradation on plant growth.

Conclusion

In this study, we addressed the issues connected with nutrient demand of biodegradable plastics biodegradation in arable soils and their negative impact on plant growth. Applying compost alone boosted the root dry biomass of lettuce, but has limited effect on aboveground biomass. Application of digestate showed, that alone, it enhanced both aboveground biomass (dry and fresh) and specific enzyme activity (β-glucosidase), indicating a beneficial impact on lettuce growth and certain soil biochemical functions. The application of P3HB alone strongly reduced both root and aboveground biomass, indicating a detrimental effect on plant growth. However, it increased activity of dehydrogenase and urease enzymes and various types of respiration, which implied enhanced microbial activity in the soil. The co-application of compost and P3HB enhanced all tested enzyme activities except for β-glucosidase, which suggested that compost can mitigate some of the negative effects of P3HB on biochemical processes in the soil. The most effective combination was digestate and P3HB, their co-application significantly countered the negative impact of P3HB on lettuce biomass and further enhanced certain enzyme activities and types of respiration. This combination appeared to be the most effective in supporting both plant growth and soil health.

In fact, both digestate and compost are valuable organic amendments that can improve soil health and plant growth, the compost seemed less advantageous for the purpose of mitigating possible adverse environmental impact of microbioplastics. Apart from lower impact of anaerobic digestion on the environment, digestate may offer additional advantages, likely due to its degree and mechanisms of organic matter decomposition, nutrient composition, moisture content, and the nature of its microbial activity. These factors make digestate particularly effective in certain agricultural contexts, though it is important to monitor conditions like salt content and potential over-fertilization. Further research is needed to optimize the use of digestate for support of bioplastic degradation in specific soil types, climates, and cropping systems to harness its full potential effectively. Nevertheless, the implications of our findings are significant for agricultural practices, particularly in managing soil health and plant growth in environments impacted by biodegradable plastics.

Furthermore, this study also confirms recent observations that integrating biodegradable plastics into agricultural practices requires a comprehensive understanding of how these materials interact with soil amendments, particularly in terms of nutrient availability, microbial activity, and water retention. These findings advocate for further research and development of best practices for the use of biodegradable plastics in different agricultural systems, ensuring that their environmental benefits are maximized while minimizing potential negative impacts on soil health and plant growth.

In summary, while biodegradable plastics offer a sustainable alternative to traditional plastics, their integration into agriculture must be managed carefully. The strategic use of organic amendments like digestate can play a vital role in this process, supporting both the degradation of bioplastics and the maintenance of healthy, productive soils. This approach is essential for the effective management of bioplastic residues, particularly in the context of global efforts to enhance sustainability in agriculture.

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Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relation-ships that could have appeared to influence the work reported in this paper.

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Biodegradation of poly-3-hydroxybutyrate after soil inoculation with microbial consortium: Soil microbiome and plant responses to the changed environment

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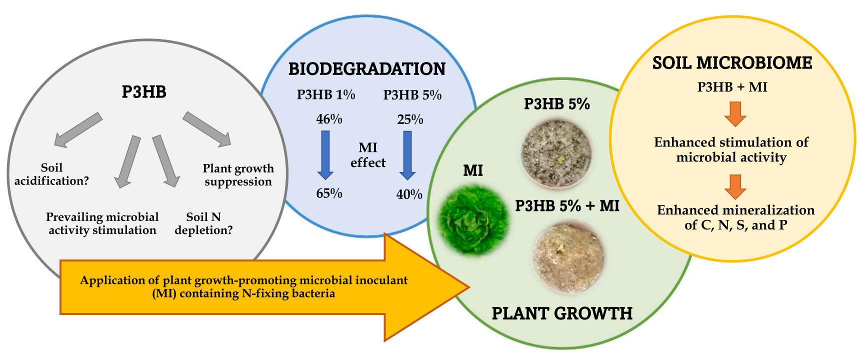
HIGHLIGHTS

- P3HB-related soil acidification was not determinative for plant growth inhibition.
- Microbial inoculant (MI) did not mitigate the adverse effect of P3HB on plant growth.
- Preferential microbial utilization of P3HB was enhanced by MI.
- MI enhanced efficiency of P3HB degradation as well as turnover of nutrients.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradable plastics play a vital role in addressing global plastics disposal challenges. Poly-3-hydroxybutyrate (P3HB) is a biodegradable bacterial intracellular storage polymer with substantial usage potential in agriculture. Poly-3-hydroxybutyrate and its degradation products are non-toxic; however, previous studies suggest that P3HB biodegradation negatively affects plant growth because the microorganisms compete with plants for nutrients. One possible solution to this issue could be inoculating soil with a consortium of plant growth-promoting and N-fixing microorganisms. To test this hypothesis, we conducted a pot experiment using lettuce (*Lactuca sativa* L. var. *capitata* L.) grown in soil amended with two doses (1 % and 5 % w/w) of P3HB and microbial inoculant (MI). We tested five experimental variations: P3HB 1 %, P3HB 1 % + MI, P3HB 5 %, P3HB 5 % + MI, and MI, to assess the impact of added microorganisms on plant growth and P3HB biodegradation. The efficient P3HB degradation,

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which was directly dependent on the amount of bioplastics added, was coupled with the preferential utilization of P3HB as a carbon (C) source. Due to the increased demand for nutrients in P3HB-amended soil by microbial degraders, respiration and enzyme activities were enhanced. This indicated an increased mineralisation of C as well as nitrogen (N), sulphur (S), and phosphorus (P). Microbial inoculation introduced specific bacterial taxa that further improved degradation efficiency and nutrient turnover (N, S, and P) in P3HB-amended soil. Notably, soil acidification related to P3HB was not the primary factor affecting plant growth inhibition. However, despite plant growth-promoting rhizobacteria and N_2-fixing microorganisms originating from MI, plant biomass yield remained limited, suggesting that these microorganisms were not entirely successful in mitigating the growth inhibition caused by P3HB.

1. Introduction

In 2019, the world's production of plastics increased to nearly 368 million metric tons (Statista, 2022). The safe and efficient disposal of used plastics and preventing their intrusion into the environment are global challenges (Zheng and Suh, 2019). Due to its toxic additives and long-term persistence in the environment, plastic pollution has become a focus point of research these days (He et al., 2018).

Biodegradable plastics can play a critical role in solving plastics disposal problems from both economic and environmental perspectives (Thakur et al., 2018). Biodegradable plastics are mainly produced from renewable resources and can be decomposed and metabolised by many organisms (Kale et al., 2015). Hence, instantly increasing the production of biodegradable plastics provides a wide range of polymers, including biopolyesters, such as polyhydroxyalkanoates (PHAs), among which the most important is polyhydroxybutyrate (PHB). Polyhydroxybutyrate is a bacterial intracellular carbon (C) and energy storage polymer that exists primarily as poly-3-HB, but other monomers – 2-HB and 4-HB – are also possible in variable lengths of monomer chains, including poly-2-HB, poly-3-HB, and poly-4-HB (Sudesh et al., 2000).

Polyhydroxyalkanoates are characterised by technologically promising properties such as biodegradability, biocompatibility, and thermoprocessibility, as well as favourable mechanical qualities (Medeiros Garcia Alcantara et al., 2020). For example, PHB biodegradability has been demonstrated in aerobic and anaerobic conditions (Nishida and Tokiwa, 1993; Sharifzadeh et al., 2009). Therefore, biodegradable polymers are commonly used as mulching films, in bands of sowing, in pots and containers and other horticulture materials and tools, or for the controlled release of agricultural chemicals (Touchaleaume et al., 2016; Volova et al., 2022). Owing to their decomposability and degradation, the micro-bioplastics may be rapidly released into agricultural soils (Fojt et al., 2020).

However, until recently, only a few studies evaluated the effect of PHB amendment on soil and plant growth. Fojt et al. (2022a) studied the effects of poly-3-hydroxybutyrate (P3HB) particles on water status in soil organic matter (SOM) under arid conditions. They concluded that contamination of sapric histosol by only partially wettable P3HB particles supported moisture loss by disrupting the SOM structure, mainly by interaction with aliphatic moieties. Similarly, Wan et al. (2019) observed that microplastics increased the rate of soil water evaporation by creating channels for water movement. Brtnicky et al. (2022) observed changes in pH of soil and soil-sand mixtures after P3HB application; P3HB-related acidification was observed when the sand content increased by ≥40 %. Zhou et al. (2021) even speculated about the possible negative impact of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)-related soil acidification on plant growth. Further, they observed an increased microbial activity and enrichment of specific bacterial taxa around the PHBV particles. A ten-month experiment by Palucha et al. (2024) demonstrated that biodegradation of P3HB may cause a priming effect in various soils, including Cambisol, Chernozem, and Phaeozem.

The effect of PHAs on plants seems to be even more significant. In summary, an adverse impact of PHBV, PHB, and P3HB on plant growth was recently observed for maize (*Zea mays* L.) (Brown et al., 2023), tomato (*Lycopersicon esculentum* Mill.) (Serrano-Ruiz et al., 2023) and lettuce (*Lactuca sativa* L. var. *capitala* L.) (Brtnicky et al., 2022), respectively. Moreover, adding PHBV ultimately resulted in wheat (*Triticum aestivum* L.) death after 25 days of exposure (Zhou et al., 2021). These negative effects of PHAs or PHB and soil degradation are valid reasons to investigate related mechanisms and influencing factors to develop appropriate management strategies for the controlled use of PHB-based plastics in agriculture.

The latest research suggests that field-weathered PHB has a stronger inhibitory effect on plants than pristine bioplastics (Serrano-Ruiz et al., 2023). To a lesser extent, PHB degradation in the soil is determined by the PHB-polymer formulation, chemical modification, and abiotic conditions (e.g., light irradiation, temperature, and mechanical disintegration). However, the length of persistence and dissipation of bioplastics in the soil is more significantly moderated by the soil's capacity and ability for their degradation. This is greatly determined by the soil biotic part, i.e., the composition of macro- (mostly earthworms) (Sanchez-Hernandez et al., 2020) and especially micro-degraders (Abou-Zeid et al., 2004; Rychter et al., 2006). The microbial community structure was referred to as the critical factor in the rapid and efficient biodegradation of PHB material in soil (Guo et al., 2010; Vogel et al., 2021). Some of the essential microorganisms with the ability to catabolise PHB belong to the groups of saprophytic fungi (Sang et al., 2002; Altaee et al., 2016) and bacteria – e.g., *Bacillus*, *Paenibacillus*, *Streptomyces*, *Arthrobacter*, *Azospirillum*, and *Pseudomonas* – (Ito et al., 1998; Manna et al., 1999; Volova et al., 2017), including rhizobacteria with plant growth-promoting effect (Bonartseva et al., 2003; Kadouri et al., 2003; Jeszeova et al., 2018).

A recent study suggested that one of the main threats during the biodegradation of P3HB in the soil is the boosted microbial catabolic activity, causing a shortage of nitrogen (N) necessary for plant growth (Brtnicky et al., 2022). This may limit the use of P3HB and, generally, all biodegradable agricultural polymers that do not contain metabolisable N in their structure. Therefore, applying a microbial consortium containing plant-beneficial bacteria (e.g., N-fixing) for promoting plant growth (Hussain et al., 2020; Yaghoubi Khanghahi et al., 2021) and degrading P3HB residues in soil (Manna et al., 1999; Jeszeova et al., 2018) can be a promising solution.

There are studies investigating the impact of soil inoculation with plant growth-promoting rhizospheric bacteria (PGPR) and fungi on poly-lactic acid (PLA) and polyethylene terephthalate biodegradation efficiency in soil planted with selected plant species and their growth (Janczak et al., 2014; Janczak et al., 2018). As far as we know, no study has been published yet evaluating the combined effects of microbial inoculation and P3HB on a soil-plant system. Therefore, this study aims to investigate the impact of simultaneously using P3HB and inoculating with microbes, the latter having potentially beneficial effects on both plant growth and P3HB degradation (such as *Azospirillum*, *Bacillus*, and *Pseudomonas*). The conclusions of the work can be an important foundation for the management of P3HB bioplastics in agriculture. We hypothesised that:

1. P3HB-related acidification is not critical for plant growth inhibition in pH-neutral soils since the pH changes recorded so far had the character of a secondary phenomenon rather than a primary cause.
2. Microbial inoculation with N-fixing microorganisms will overcome the N shortage caused by P3HB degradation and, thus, will mitigate the adverse effect of P3HB on plant growth.
3. P3HB degradation in soil will be enhanced by microbial inoculation.
4. Microbial inoculation will further enhance increased microbial activity as an indicator of nutrient turnover coupled with P3HB degradation.

2. Material and methods

2.1. Experiment design

The silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification) was collected from a depth of 0–15 cm near the town of Troubsko, Czech Republic (49°10′28″ N 16°29′32″ E). The growth substrate used for the pot experiment was obtained by mixing (1:1, w:w) a fine quartz sand (0.1–1.0 mm; ≥95 % SiO_2) with arable soil sieved through a sieve with a mesh size of 2 mm (Hammerschmiedt et al., 2022). The chemical composition of the soil was as follows: total C 14.0 $g{\cdot}kg^{-1}$, total N 1.60 $g{\cdot}kg^{-1}$, P 0.097 $g{\cdot}kg^{-1}$, S 0.145 $g{\cdot}kg^{-1}$, Ca 3.26 $g{\cdot}kg^{-1}$, Mg 0.236 $g{\cdot}kg^{-1}$, K 0.231 $g{\cdot}kg^{-1}$, and pH ($CaCl_2$) 7.3. Water holding capacity of soil was determined using a pressure plate method.

Poly-3-hydroxybutyrate powder (ENMAT Y3000) was obtained from TianAn Biologic Materials Co., Ltd. (Ningbo City, China). The specific weight was 1.25 $g{\cdot}cm^{-3}$, crystallinity approximately 49 % (Procházková et al., 2024). Other parameters of the P3HB were summarised by Melcova et al. (2020) and provided by the manufacturer. The particles in the powder were spherical, ranging from 200 nm to 80 m and with a contact angle of ≈70°–81°, making them slightly hydrophobic (Fojt et al., 2022a). According to the provider, the powder did not contain copolymers. Also, the recent work of Procházková et al. (2023) in their ecotoxicological study revealed that the powder and its water extract were not toxic for aquatic plants except for a very high dose, which showed a small effect in case of water extract.

Poly-3-hydroxybutyrate content in agricultural soils with P3HB mulching sheets can range from approximately 0.5 % to 1.5 %, but it can be probably even higher in the future (Palucha et al., 2024). Therefore, 1 kg of growth substrate was mixed with a defined dose (0 %, 1 %, and 5 % w/w) of P3HB to prepare experimental variants and filled into experimental plastic pots (volume 1 L, top diameter 11 cm, bottom diameter 9 cm, and height 13 cm). Inoculated variants were enriched with 80 μL of the commercially available microbial inoculant (MI) Rewital Biogen Pro+ (BIO-GEN, Poland) diluted in 50 mL of deionised water. The composition of Rewital (from the provider) is reported in Table 1. Variants without inoculation were watered only with 50 mL of deionised water. To summarise, the following experimental variants were prepared: control, MI, P3HB 1 %, P3HB 1 % + MI, P3HB 5 %, P3HB 5 % + MI. Each variant was readied in three replicates (pots).

The pot experiment with crop lettuce (*Lactuca sativa* L. var. *capitata* L. cv. Brilliant) took place in growth chamber Climacell Evo (BMT, Czech Rep.) under the following controlled conditions: white LED lighting, intensity 20 klx; photoperiod 12 h; temperature 18 °C/22 °C (night/day) and relative air humidity 70 %. Lettuce seeds were pregerminated on wet filter paper for two days and then sown to a depth of approximately 2 mm in the experimental pots. After sowing, each pot was watered with 50 mL of distilled water. The 10-day-old seedlings were reduced from five to a single most robust plant per pot. Pot placement in the growth chamber was randomised. Soil moisture was controlled gravimetrically, and the water content was maintained (two to three times a week) at approximately 60 % of the water-holding capacity during the experiment. Specifically, 1 g of soil was regularly sampled and the water content was determined gravimetrically. Accordingly, calculated volume of water was dosed to the pot to keep 60 % of soil water holding capacity. Pots were variably rotated once a week to maintain homogeneity of growing conditions. The plants were harvested eight weeks after sowing.

The lettuce shoots were cut off at ground level, and the roots were gently cleaned off of soil and washed with water. Lettuce shoots and roots were dried at 60 °C to constant weight; dry aboveground biomass (AGB) and root biomass were determined gravimetrically by weighing on analytical scales.

2.2. Soil analysis

After harvesting the lettuce, a mixed soil sample was taken from each pot. The soil samples were homogenised by sieving through a 2 mm mesh sieve. Air-dried samples were analysed for pH (ISO 10390:2005). Freeze-dried samples were used for analysing enzymatic activities: β-glucosidase (GLU), urease (Ure), *N*-acetyl-β-D-glucosaminidase (NAG), arylsulfatase (ARS), and phosphatase (Phos) (ISO 20130:2018). The samples stored at 4 °C were used for the determination of dehydrogenase activity (DHA) (Doi and Ranamukhaarachchi, 2009), soil basal respiration (BR), and substrate-induced respirations (IR) – D-glucose (Glc-IR), D-trehalose (Tre-IR), *N*-acetyl-β-D-glucosamine (NAG-IR), L-alanine (Ala-IR), L-lysine (Lys-IR), and L-arginine (Arg-IR) (Campbell et al., 2003) – using MicroResp® device (The James Hutton Institute, Scotland).

The share of biodegraded P3HB was determined as follows (Fojt et al., 2022b): the homogenised soil samples were air-dried and exposed to 43 % relative humidity to ensure uniform conditions for all soils. After two weeks, the samples were analysed using thermogravimetry analyser Q550 from TA Instruments (Delaware, USA). Approximately 200 mg of soil was placed on an alumina crucible and heated from 25 °C to 650 °C under a dynamic atmosphere of air (flow rate 100 mL min^{-1}). TRIOS software (TA Instruments) was used to evaluate the mass loss between 200 °C and 300 °C, where the residual P3HB thermally degrades (Fojt et al., 2022b). Soil from each pot was analysed in triplicate. The share of biodegraded P3HB was calculated as follows: thermogravimetrically recorded mass losses for P3HB-free samples with and without MI were subtracted from recorded mass losses of respective P3HB-amended variants (either 1 % or 5 %) in the range 200 °C–300 °C as

Table 1
Taxonomical composition of microbial consortium (10^8 $CFU{\cdot}mL^{-1}$) in the microbial inoculant Rewital Biogen Pro+.

Species	Gram	Family	Oxygen demand	Characteristics
Azospirillum lipoferum	Positive	*Rhodospirillaceae*	Aerobic	N_2-fixation, PGPR
Lactobacillus acidophilus	Positive	*Lactobacillaceae*	Microaerobic	Produces lactic acid
Lactobacillus plantarum	Positive	*Lactobacillaceae*	Microaerobic	Producer of lactic acid
Bacillus subtilis	Positive	*Bacillaceae*	Aerobic	PGPR, P-solubilisation
Pediococcus acidilactici	Positive	*Lactobacillaceae*	Microaerobic	Producer of lactic acid
Pseudomonas fluorescens	Negative	*Pseudomonadaceae*	Aerobic	PGPR, and producer of lipases and proteases
Ruminococcus albus	Positive	*Ruminococcaceae*	Anaerobic	Rumen microorganism
Cellulomonas cellulans	Positive	*Cellulomonadaceae*	Aerobic	Cellulose hydrolysis
Azotobacter vinelandii	Negative	*Pseudomonadaceae*	Aerobic	PGPR, N_2-fixation

More details are available at: https://www.procam.pl/landing/biogen-rewital-pro/.

demonstrated by Palucha et al. (2024). For example, the residual P3HB was determined as follows: 1 % P3HB variants gave 0.54 ± 0.06 %, which, divided by the number of days in the experiment, gives an averaged P3HB mass loss rate of about 0.08 ± 0.01 g·kg^{-1}·day^{-1}. Notably, as demonstrated by Palucha et al. (2024), the priming effect of P3HB on soil occurs after complete plastic degradation; therefore, possible mass loss of SOM in the range 200 °C–300 °C was not considered during the calculations.

2.3. Sequencing analysis of the soil microbiome

The total microbial DNA was extracted from 0.5 g of freeze-dried soil sample using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA). Then, DNA was used to amplify specific regions of the rRNA genes of fungi ITS2 (18S) and bacteria V3–V5 (16S) by utilising the primers F357 (5′-CCTACGGGAGGCAGCAG-3′) and R926 (5′-CCGYCAATTYMTT-TRAGTTT-3′), or ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′), respectively, with barcoding and the universal overhang. Illumina sequencing adaptors were introduced in the second PCR, all in accordance with the standard instructions (Illumina, 2013). The products were evaluated by agarose electrophoresis, quantified with a fluorimetric, AccuGreen™ High Sensitivity dsDNA Quantitation Kit (Biotium, Inc., CA, USA) and pooled into a library. Sequencing was conducted on a MiSeq unit (Illumina, Inc., CA, USA) running the reagent kit v2 and paired-end 250 nt reads in an external laboratory (SEQme s.r.o., Czech Republic). To decide whether the bacteria strains introduced in MI could influence the procaryotic community's composition, the available sequences were searched for the genera present in MI and their relative abundances were compared among the variants.

2.4. Data processing and statistical analysis

Data processing and statistical analyses were performed using R, version 3.6.1. (R_Core_Team, 2020). Principal component analysis (PCA) and one-way analysis of variance (ANOVA) type I (sequential) sum of squares at a 5 % significance level (Zar, 1984) methods were used for characterising the relationship between the variants and selected soil properties. To detect the statistically significant difference among factor level means, Tukey's honestly significant difference (HSD) test and 'variant contrast' were used to calculate factor level means for each variant. The results were graphically presented with a Rohlf biplot for standardised PCA. Pearson correlation analysis was performed to measure the linear dependence between soil properties. Pearson correlation coefficient was interpreted as follows: $0.0 < r < 0.3$: negligible correlation, $0.3 < r < 0.5$: low correlation, $0.5 < r < 0.7$: moderate correlation, $0.7 < r < 0.9$: high correlation, and $0.9 < r < 1.0$: very high correlation (Hinkle et al., 2003).

The data from the soil microbiome sequencing analysis were further processed with the DADA2 R package (Callahan et al., 2016) and visualised by the phyloseq R package (McMurdie and Holmes, 2013) and microeco R package (Liu et al., 2021). Taxonomy was assigned for the bacteria according to the SILVA 132 SSU NR 99 reference database (Quast et al., 2012) and the 8.3 release of the UNITE reference database (Kõljalg et al., 2020) for fungi.

The α-diversity of the procaryotic and fungal microbial communities was determined by the Simpson and Shannon diversity indexes.

3. Results

3.1. Influence on pH

Microbial inoculant and a low dose of P3HB did not affect soil pH. However, their combination, as well as a high dose of P3HB applied alone, significantly decreased pH (Table S1). There was a noticeable decrease in pH in variants with 5 % P3HB, where pH decreased from 7.34 ± 0.01 to 6.83 ± 0.02 (without MI) and 6.72 ± 0.03 (with MI).

3.2. Influence on plant growth

Microbial inoculation solely stimulated AGB and root plant biomass growth (Fig. 1a, b; Table S2). However, the presence of P3HB in the soil significantly suppressed biomass production in all variants, including that with MI.

3.3. Influence on microbial activity

The key studied indicators of soil microorganisms' degradation activity were DHA and respirations (Fig. 2). Dehydrogenase activity increased with an increasing P3HB dose in both uninoculated and inoculated variants (Fig. 2a). Co-application of MI with P3HB at a high dose indicated a synergic positive effect on DHA, undetectable at a low P3HB dose.

Soil BR and most IRs (Fig. 2b–h) demonstrated similar trends as DHA: positive direct dependence on P3HB dose (except for Tre-IR and Arg-IR at a 1 % P3HB dose). In addition, the stimulating effect of P3HB on respiration values increased even with inoculation. When used solely, MIs showed a positive impact on BR and a negative effect on Glc-IR and Tre-IR, while IRs by the remaining substrates were unaffected.

Relative average values of DHA, BR, and all types of IRs were higher in combined than in P3HB-only variants (Table 2). The relative positive contribution of MI on the enhancement of P3HB-stimulated degradation activity was at a similar level at both doses of P3HB except for DHA and Arg-IR (the last two rows in Table 2). The results suggested that MI stimulated degradation activity occurs more efficiently at high P3HB doses. Moreover, MI was more beneficial than the 1 % P3HB amendment for Arg-IR. Similar trends and relationships between these characteristics were also reflected statistically as DHA and all respiration types correlated positively with DHA and among each other (Figs. S1 and S2).

The combination of high P3HB dose with MI increased activity of all enzymes involved in C, N, S, and P cycle with respect to control and with respect to the low dose P3HB addition with or without MI (Fig. 3a–e). MI significantly increased the activity of Phos, ARS and NAG at the high P3HB dose, of GLU and NAG at the low dose, and only of ARS for the control without P3HB addition. Conversely, 1 % P3HB decreased GLU values, with MI suppressing this negative effect (Fig. 3a). *N*-acetyl-β-D-glucosaminidase activity increased in a P3HB dose-dependent manner, and this was further enhanced by MI (Fig. 3d). Poly-3-hydroxybutyrate caused a dose-dependent increase in Ure activity with no effect on MI (Fig. 3e).

Arylsulfatase was stimulated by both P3HB and microbial inoculation in original soil (Fig. 3c). Unlike the previous microbial properties, the activity of Phos in the solo P3HB 5 % variant was significantly decreased (Fig. 3b), while the only positive effect was reached by a high dose of P3HB supplemented with the MI. Phos correlated only weakly with other properties (Fig. S1).

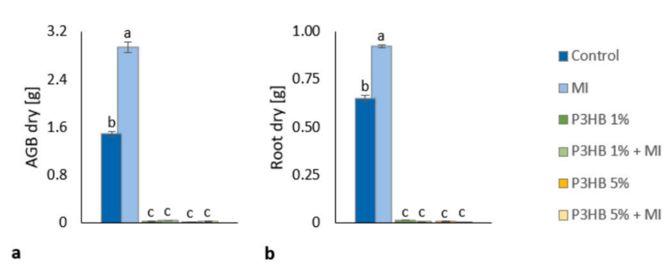

Fig. 1. Lettuce (a) aboveground (AGB) and (b) root plant dry biomass of the variants amended with different doses of poly-3-hydroxybutyrate (P3HB) and/or with microbial inoculant (MI). The lowercase letters indicate significant differences at $p \leq 0.05$.

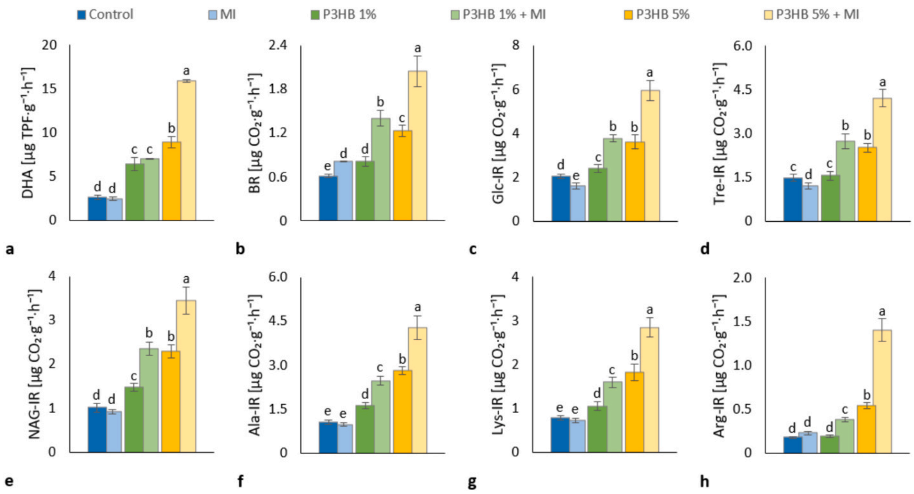

Fig. 2. Soil (a) dehydrogenase activity, (b) soil basal respiration, and respiration induced by (c) D-glucose, (d) D-trehalose, (e) *N*-acetyl-β-D-glucosamine, (f) L-alanine, (g) L-lysine, and (h) L-arginine in the variants amended with different doses of poly-3-hydroxybutyrate (P3HB) and/or with microbial inoculant (MI). The lowercase letters indicate significant differences at $p \leq 0.05$.

Table 2
Relative average values of dehydrogenase activity (DHA), basal respiration (BR), and all types of induced respirations (IR). The relative values were calculated as a ratio between the absolute values of each property in the amended variant and control.

Ratio between the variants	DHA	BR	Glc-IR	Tre-IR	NAG-IR	Ala-IR	Lys-IR	Arg-IR
1 % P3HB: Control	2.38	1.31	1.17	1.05	1.46	1.52	1.33	1.11
1 % P3HB + MI: Control	2.60	2.26	1.83	1.83	2.35	2.35	2.01	2.11
5 % P3HB: Control	3.33	1.98	1.75	1.69	2.28	2.65	2.32	3.00
5 % P3HB + MI: Control	5.90	3.29	2.88	2.83	3.43	4.06	3.61	7.78
MI: Control	0.92	1.31	0.78	0.80	0.93	0.94	0.91	1.28
1 % P3HB + MI: 1 % P3HB	1.09	1.73	1.56	1.74	1.61	1.55	1.51	1.90
5 % P3HB + MI: 5 % P3HB	1.77	1.66	1.65	1.67	1.50	1.53	1.56	2.59

3.4. *Influence on microbial community diversity and taxonomic composition*

The procaryotic community responded to both additives and their combinations (Figs. 4a, S3a). Although MI and 1 % P3HB amendment had an almost identical weak effect, their combination and 5 % P3HB dose showed a relevant change in the community.

Families *Nitrososphaeraceae*, *67-14*, and *Gaiellaceae* were negatively affected by P3HB (Fig. S3a). On the contrary, *Oxalobacteraceae*, *Sphingomonadaceae*, and *Comamonadaceae* were stimulated by it. Relative abundances of *Pseudomonadaceae*, *Nocardiadaceae*, *Microbacteriaceae*, and *Caulobacteriacea* increased after the combination of P3HB and MI. At the genera level, mainly *Azospirillum* and *Azotobacter* were enhanced at both combinations of P3HB and MI but usually without statistical significance (Fig. 5a, c, e), with significantly reduced abundance for 5 % P3HB + MI only (Fig. 5b) and with significantly increased abundance for 5 % P3HB + MI only (Fig. 5d and f).

Fungi communities responded more strongly even at the low P3HB dose (Figs. 4b, S3b). Saprophytic fungi *Exophiala* and *Tetracladium*, related to the decay of organic matter, were highly stimulated by P3HB. On the contrary, the relative abundances of the originally common *Gibellulopsis* and *Fusarium* significantly decreased. *Pseudeurotium* and *Cyberlindnera* were stimulated only by the variant 5 % P3HB with MI.

The Simpson index showed a slight decrease in diversity only in the variant with 5 % P3HB supplemented with the MI (Fig. S4a, c). The Shannon index indicated similar results (Fig. S4b, d). Both bacteria and fungi communities followed a similar trend, with a slight diversity decrease in response to the P3HB increase.

3.5. *Residual P3HB*

Fig. 6 reports exemplary thermogravimetry records. The presence of residual P3HB caused a sharp mass loss in the temperature interval 200 °C–300 °C compared to the P3HB-unamended soils, where the mass loss was only moderate. As mentioned in the Experiment design section of this work, in this temperature interval, the degradation of both residual P3HB and labile fraction of SOM occurs, which was used to determine the residual P3HB in soils. Accordingly, the variants P3HB 1 %, P3HB 1 % + MI, P3HB 5 %, and P3HB 5 % + MI exerted following losses of P3HB (per a kg of soil substrate, in average) after 56 days of the experiment: 4.6 ± 0.6 g, 6.5 ± 0.9 g, 12.4 ± 1.1 g, and 21.2 ± 1.3 g, respectively, which equalled approximately 46 %, 65 %, 25 %, and 40 % of the starting amount of added P3HB. Furthermore, the 1 % P3HB variants without and with MI resulted in an averaged P3HB mass loss rate of approximately 0.08 ± 0.01 and 0.12 ± 0.03 $g \cdot kg^{-1} \cdot day^{-1}$, respectively. The 5 % P3HB produced an averaged P3HB mass loss rate of nearly 0.22 ± 0.01 and 0.38 ± 0.03 $g \cdot kg^{-1} \cdot day^{-1}$ for soils without and with MI, respectively. Therefore, variants with microbial inoculation exhibited more intensive degradation than those without microbial inoculation.

4. Discussion

4.1. *Influence on pH and plant growth*

Zhou et al. (2021) speculated that PHBV induced soil phytotoxicity due to the acidification effect of its degradation. The change in pH observed for P3HB (Brtnicky et al., 2022) highlights its potential significance for bioplastics impact assessment. However, although these authors rejected the hypothesis of the phytotoxic effect of PHB-related acidification, they could not directly confirm it.

Our results indicated that soil acidification caused by a high dose of

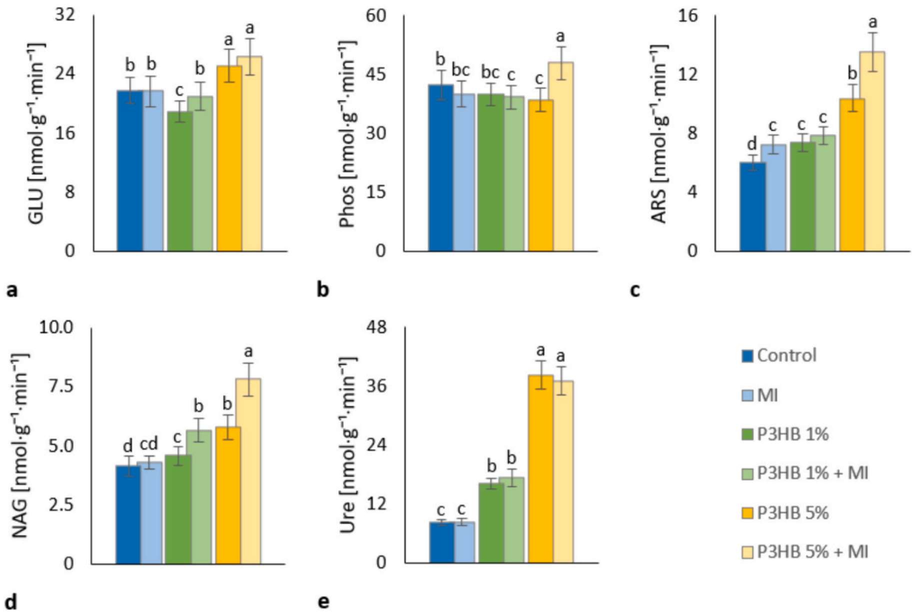

Fig. 3. Soil enzyme activities of (a) β-glucosidase, (b) phosphatase, (c) arylsulfatase, (d) *N*-acetyl-β-D-glucosaminidase and (e) urease in the variants amended with different doses of poly-3-hydroxybutyrate (P3HB) and/or with microbial inoculant (MI). The lowercase letters indicate significant differences at $p \leq 0.05$.

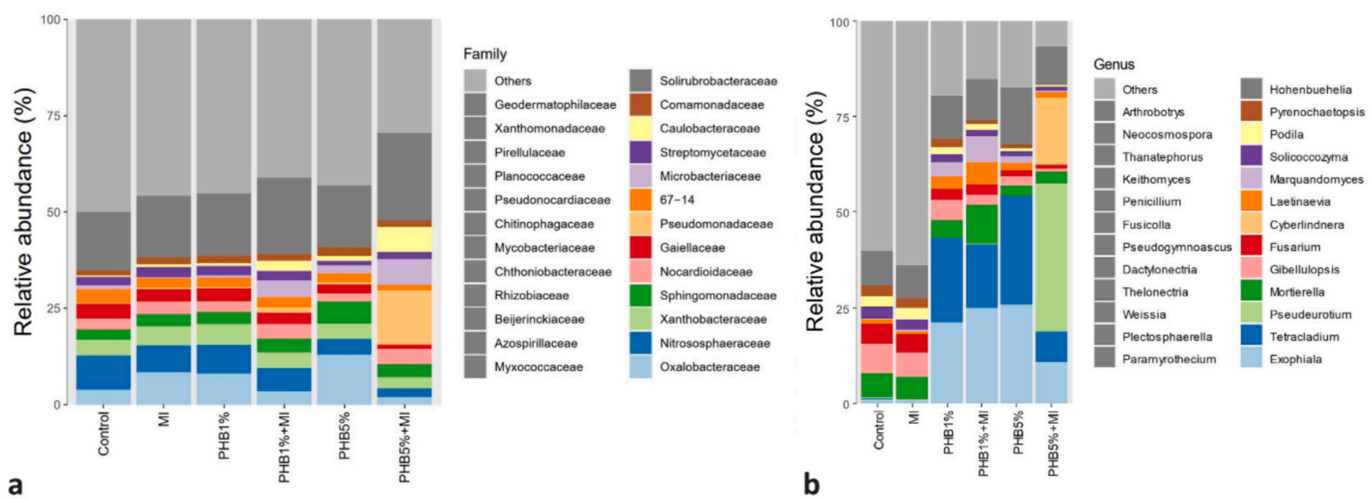

Fig. 4. Relative abundance of (a) procaryotic taxa at the family level and (b) fungi taxa at the genus level in the variants amended with different doses of poly-3-hydroxybutyrate (P3HB) and/or with microbial inoculant (MI). Driving families or genera, respectively, are highlighted in separate colours, and less abundant ones are grouped in grey.

P3HB can be intensified when co-applying P3HB with MI (Table S1) although under specific conditions, MI may mitigate this problem (Msimbira and Smith, 2020). The increase in the production and release of 3-hydroxybutyric acid due to the increased activity of P3HB-degrading microorganisms may be the reason. However, as plant growth deteriorated even in the 1 % P3HB variant with unchanged neutral pH, the first hypothesis was confirmed that the observed acidification is not responsible for plant growth inhibition in pH-neutral soils.

Although acidification probably did not directly affect the soil-plant system (Figs. 1b, c, 2, 3) due to originally pH-neutral soil, it may be a threat in acidic soils. It can affect soil quality in the long term, disrupt the supply of nutrients to plants due to leaching, and reduce crop yields (Goulding, 2016).

With the first hypothesis confirmed, the other results seem to support the assumptions of Brtnicky et al. (2022) that the microbial processes coupled with the degradation of P3HB might lead to nutrient depletion in soil and have a substantial adverse effect on lettuce growth and yield (Figs. 1b, c, S1, S2, S5). As the microbial inoculation with PGPR with potential for nitrogen fixation (*A. lipoferum* and *A. vinelandii*) and solubilisation of phosphorus (*B. subtilis*) did not mitigate the adverse effect of P3HB (Fig. 1b, c), the second hypothesis was rejected. The alternative explanation is also may be the lack of another micro-nutrient, which related-enzymes were not analysed in this work. This conclusion aligns with the results of Zhang et al. (2023) who observed the nitrogen deficiency in soil during the biodegradation of biodegradable microplastics such as P3HA, polybutylene succinate and polylactic acid. The biodegradation was accompanied by priming effect and authors concluded that stronger degradability caused greater priming effect in soil. The conclusions of our work of those of Zhang et al. (2023) are also supported by other authors investigating the effect of labile, fast biodegradable substrates with high C:N ratio, on plant growth. For example, Jonasson et al. (1996) studied the effect of slowly degrading sawdust and microbiologically labile sucrose amendments in growth of herbs and shrubs. In the case of sucrose, the authors observed a reduction in soil inorganic N

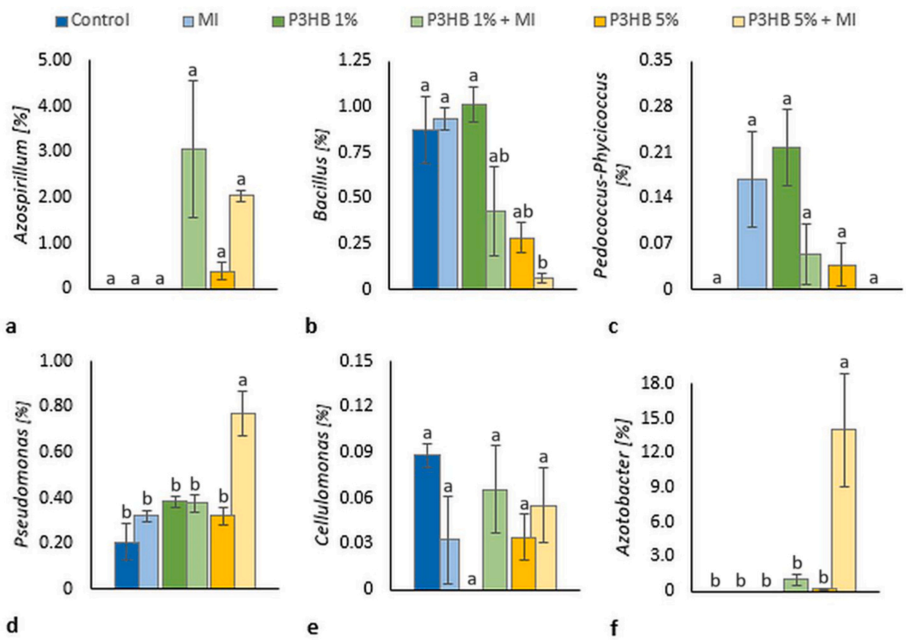

Fig. 5. Relative abundance of the genera present in the microbial inoculant (MI) in the variants amended with different doses of poly-3-hydroxybutyrate (P3HB) and/or with MI. The lowercase letters indicate significant differences at $p \leq 0.05$.

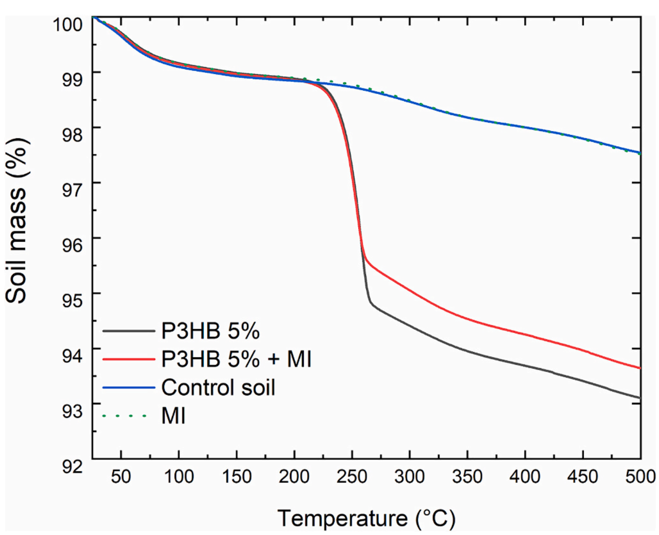

Fig. 6. Thermogravimetry records of control, inoculated soil (MI), and soils amended with 5 % P3HB (without/with MI). The mass loss ≤200 °C corresponds to moisture evaporation, and >200 °C corresponds to P3HB and soil degradation.

and P and an increase in N and P in the microbial biomass while the biomass production of herbs declined to about one third compared to control soils. In addition, a declination of N plant biomass was observed. These effects were not observed in sawdust emended variants, which indicates that the effect may also indirectly depend on the rate of amendment biodegradation. As the P3Hb is a fast degrading bioplastic, the negative may be related also to the fact that fast degradation connected with fast proliferation of microorganisms (i.e. fixation of nutrients). This can negatively impact the nutrient balance in soil resulting in a plant stress. Similar results observed also Schmidt et al. (1997) who studied the effects of addition sucrose, fructose and glucose combined with high and low levels of N and P on growth of *Festuca vivipara*. In all variants, sugar addition reduced plant growth. The inhibition of plant growth was lower in the soil, which was sterilized prior to the experiment. The authors, concluded that this was likely due to a slow recovery of the microbial populations immobilizing the nutrients.

Therefore, the main reason of inhibition of plant growth is the deficiency of micronutrients. Nevertheless, influence of other stressing factors, potentially less important than micronutrients, cannot be rejected. Fast biodegradation of labile material can lead to the depletion of oxygen in soil with a negative impact on root system, as demonstrated on biodegradation of plant residues (Siedt et al., 2023). However, the negative effects of labile materials were observed under both aerobic (Schmidt et al., 1997) and anaerobic conditions (Adachi et al., 1997), therefore, this effects needs to be further investigated. Next, an increased water evaporation or drain-off in the P3HB-amended soil because P3HB is an insoluble material with poor wettability (Fojt et al., 2022a). In particular, Niu et al. (2016) reported that the evaporation coefficient increased with the increment of amended residual plastic film in the soil, indicating a decreased water-holding capacity. Wan et al. (2019) observed an increased rate of soil water evaporation due to plastics in soil. Fojt et al. (2022a) stated that adding P3HB negatively influenced the strength of water binding. Nevertheless, the pot soils were regularly measured for their water content and irrigated accordingly in this work. As a result, the impact of stress caused by moisture shortage cannot be investigated easily, and further research is required to verify this hypothesis.

Other factors to consider may include the ecotoxicity of degradation products. As previously mentioned, the biodegradation of P3HB produces 3-hydroxybutyric acid. As shown in previous studies, incorporating 3-hydroxybutyric acid into *Linum usitatissimum* L. resulted in changes to DNA de-/methylation patterns, potentially impacting the expression of genes in the phenylpropanoid pathway (Mierziak et al., 2020), which can result in a change in plants respond to abiotic stresses (Sharma et al., 2019). Furthermore, there were reported also negative impacts of P3HB on germination, emergence, and seedling survival in the case of transgenic *Camelina sativa*. Also, the inhibitory effect of butyric acid was reported for rice planted under anaerobic conditions (Rao and Mikkelsen, 1976). On the contrary, some other authors reported that 3-hydroxybutyric acid can be utilized by soil microorganisms which would decrease its harmful effect on plant growth (Kozlovski et al., 1999; Jendrossek and Handrick, 2002). However, as aforementioned, the negative impacts of biodegradation have been observed for a wide range of labile substrates, which do not produce 3-hydroxybutyric acid, but other metabolites. Therefore, the effect of 3-hydroxybutyric acid cannot be ruled out, but it is probably not the main reason.

The efficiency of P3HB degradation depends on the state of the plastisphere (Peng et al., 2022) and the availability of nutrients, water, and oxygen (Brtnicky et al., 2022). A higher rate of P3HB degradation (per unit mass) was observed in the variants initially containing 5 % P3HB compared to those with 1 % P3HB. This indicates faster degradation both in the presence and absence of MI. The fastest degradation in 5 % P3HB compared to 1 % P3HB samples can be explained by a Monod model describing the growth of microorganisms and substrate use (Alvarez-Ramirez et al., 2019).

The third hypothesis has been validated regarding the findings demonstrating a more pronounced P3HB degradation in microbial inoculation (as discussed in Section 3.4). Other studies reported a similar percentage of bioplastics losses via biodegradation, but after a longer time: 48.5 % of PHA after approximately 40 weeks (Gomez and Michel, 2013) and 55 % of PHB after approximately 15 weeks under the effect of Siberian soil from under the larch (Boyandin et al., 2012). However, the experiments reported in the present work differed in conditions and methods for determining biodegradability. In particular, Gomez and Michel (2013) used closed jars without regular irrigation. In contrast, Boyandin et al. (2012) conducted the experiment under natural conditions, which included variations in temperature and water regime (as discussed later). Therefore, the differences in those results clearly demonstrate that the variability of conditions is decisive for the rate of bioplastics biodegradation.

4.2. *Influence on microbial activity*

4.2.1. *Microbial activity coupled with soil C dynamics*

The dose-dependent increase in degradation activity with increased P3HB addition (Table 2) indicates that the C in P3HB was the preferentially utilized energy source (Brtnicky et al., 2022). The addition of labile C substrate to soil is frequently accompanied by a general increase in SOM turnover (Zhou et al., 2020). The lower than expected stimulation of β-glucosidase activity (Fig. 3a) aligns with Zhou et al.'s (2021) presumption that PHBV stimulates the breakdown of other common soil polymers (i.e. cellulose).

Enrichment of soil microbiome with bacterial species from MI was expected to positively affect C mineralisation and P3HB degradation as different microbial groups participate in the latter (Bonartseva et al., 2003; Rizzarelli et al., 2004). When used in isolation, MI stimulated only BR in all variants (Fig. 2) and for DHA for the 5 % P3HB variant, with no benefit to the C mineralisation rate (Table 2), probably because it specialises in a specific soil substrate. As the ability to degrade the added substrates for IR was not enhanced by MI, they are possibly not diverse enough to cover a wide range of degradation functions. In contrast, P3HB and MI co-application enhanced C mineralising activities expressed by the respirations at both doses of P3HB and by GLU and DHA at one (Figs. 2, 3). This finding originates from the increased abundance of potentially P3HB-degrading microorganisms (Figs. 4, 5d) and the wide availability of C sources represented by P3HB particles. These results confirmed our third and fourth hypotheses. Previously, a similar increase in the bioplastic degradation in soil inoculated with microorganisms was reported for poly-butylene succinate (Abe et al., 2010), PLA (Janczak et al., 2018), and the plasticiser di(2-ethylhexyl) phthalate (Bai et al., 2020) mainly due to the application of specific species adapted to the given conditions. The specific adaptation to the given conditions was also decisive in this case (Figs. 4, 5) and was mainly conditioned by the content of P3HB.

4.2.2. *Microbial activity coupled with soil N dynamics*

The positive correlation of N mineralisation indicators such as NAG, Ure, NAG-, Ala-, Lys-, and Arg-IR with C mineralisation indicators (DHA, GLU, and respiration types; Figs. S1, S2) suggested that similar mechanisms as described above (which boosted C mineralisation) were involved in the enhancement of soil N mineralisation potential. However, in the absence of an additional N source, the availability of readily utilisable P3HB likely promoted N mining and coupled SOM degradation. This probably resulted in the increased release of SOM-bound N, which was associated with the activity of soil fungal biomass (indicated by NAG and NAG-IR), as reported previously (Brtnicky et al., 2022). This supports our fourth hypothesis.

N-acetyl-β-D-glucosaminidase highly correlated with Ure (Fig. S1), P3HB presence in soil-derived urease increase, but without any further accessory effect of MI (Fig. 3e). It was hypothesised that the microbial consortium containing, among others, N_2-fixing bacteria *Azotobacter vinelandii* and *Azospirillum lipoferum* (Table 1), could contribute to higher rates of N_2 assimilation in the soil and subsequent increased turnover of bacterial biomass-related N. However, despite the indicated stimulation of *Azospirillum* and *Azotobacter* abundance by combined P3HB and MI (Fig. 5a, f), microbial inoculation probably increased only fungal biomass-accompanied N mineralisation (Fig. 3d).

Microbial inoculant-derived increased soil microbial abundance and P3HB-enhanced microbial degradation likely led to a higher demand for N. This could stimulate competition for this element between plants and microbes and subsequently cause a significant deficit of N available to plants. In conclusion, we may speculate whether the MI did not exacerbate the unavailability of N for plants. However, the results do not definitively confirm or reject this speculation (Fig. 1).

4.2.3. *Microbial activity coupled with soil S and P dynamics*

The significant positive relations between ARS and DHA, GLU, BR, and IRs (Figs. S1, S2) suggested that increased P3HB-derived C mineralisation was coupled with higher S mineralisation in the soil, as already reported by Brtnicky et al. (2022). Higher P3HB doses further activated and multiplied soil microbiome and promoted higher demand for S in the available form for both plants and microbes. Niknahad Gharmakher et al. (2008) referred to S mineralisation as being predominantly driven by heterotrophic microbial activity in the soil. The positive effect of microbial inoculation on ARS (Fig. 3c) confirmed an enhanced nutrient transformation rate in the MI-treated soil, in line with the fourth hypothesis.

However, the Phos results (Fig. 3b) were not entirely in line with the assumption, as its values demonstrated indirect dependence on P3HB doses. This may indicate that the content of available organic P in the soil was insufficient and was readily exhausted due to the increased demand under more induced nutrient acquisition at 5 % P3HB dose. The presumed low abundance of phosphate-solubilising bacteria accompanying the shift in the microbial community in the P3HB 5 % variant (Fig. 4) was indicated by the presence of *Bacillus* (Fig. 5b). However, this trend was likely counteracted by the MI enriching the soil microbiome with the progressive P-solubiliser *Pseudomonas* (Fig. 5d).

4.3. *Influence on microbial community diversity and taxonomic composition*

Despite the changes in the communities induced by P3HB and MI (Figs. 4, S3), α diversity was primarily stable, indicating the limited effect of the amended doses on microbial diversity (Fig. S4).

Changes in the microbiome associated with P3HB were likely related to nutrient availability. Poly-3-hydroxybutyrate-related decrease of relative abundancies of *Nitrososphaeraceae*, *Xanthobacteriaceae*, and *Gaiellaceae* (Fig. S3a), among others, could reflect the reduced availability of N and P depleted during P3HB biodegradation (Wang et al., 2022). The increase in relative abundances of several taxa (Figs. 4, S3) is possibly linked to P3HB-related C. For example, *Tetracladium* was abundantly present in the soil contaminated with biopolyester poly (butylene succinate-*co*-adipate) and was recognised as involved in its degradation (Purahong et al., 2021). Black yeast representatives, such as *Exophiala oligosperma* R1, *E. xenobiotica*, or *E. jeanselmei*, were shown to degrade various C substrates (Rustler and Stolz, 2007; Radwan et al., 2020).

The co-application of P3HB with MI had specific responses (Figs. 4, S3). For example, the abundances of *Pseudomonadaceae*, *Nocardiadaceae*, *Microbacteriaceae*, and *Caulobacteriacea* were enhanced. These taxa are important plant symbionts (Duan et al., 2021); however, at least some can also be labelled as copiotrophic, profiting from the readily available C substrate (Chiba et al., 2021).

The results of microbial community diversity and taxonomic composition indicate the need for a study devoted to a deeper insight into the changes in the soil microbiome and related processes in response to P3HB.

5. Conclusions

The pot experiment established efficient P3HB degradation, with the degradation rate directly dependent on the amended dose of the bioplastic. The added P3HB enhanced soil microbial activity, indicating a higher mineralisation rate for C, N, and S caused by increased SOM turnover due to higher demand for nutrients in the P3HB-amended soil. Most microbial activity indicators were significantly positively related to increasing the dose of P3HB and the inoculation with the microbial consortium. Microbial inoculants provided functionally specific bacterial taxa, which partially positively responded to P3HB in soil and further enhanced the degradation efficiency as well as the turnover of nutrients (N, S, and P) in P3HB-amended soil. Higher P3HB concentrations strongly deteriorated lettuce growth, which was not ascribed to P3HB-related soil acidification. The plant growth inhibition was not mitigated either by PGPR with expected N availability support. The extensive use of P3HB in agriculture (e.g. in the form of mulching sheets) remains a threat even with microbial inoculation of soil aimed at reducing P3HB-related adverse effects. Nevertheless, the results indicate the potential of using microbial inoculation to decontaminate P3HB-burdened agricultural soils. However, there are still risks such as i) further regeneration processes connected to restoration of natural soil microbiome and ii) insufficient nutrient content (due to accelerated run-off), necessary for the sustainability of agricultural production, as soils disturbed like this exhibit a persistent reduction in yields. In future research, it is necessary to focus in more detail on (i) soil nutrients and their availability in soil affected by P3HB and (ii) the long-term development of the soil microbiome affected by P3HB and related time necessary for complete P3HB degradation.

CRediT authorship contribution statement

Martin Brtnicky: Writing – review & editing, Writing – original draft, Project administration, Methodology, Conceptualization. **Vaclav Pecina:** Writing – review & editing, Writing – original draft, Supervision. **Jiri Kucerik:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Tereza Hammerschmiedt:** Writing – original draft, Visualization, Conceptualization. **Adnan Mustafa:** Writing – review & editing, Writing – original draft, Resources. **Antonin Kintl:** Writing – review & editing, Writing – original draft, Resources, Project administration. **Jana Sera:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Marek Koutny:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Tivadar Baltazar:** Writing – review & editing, Writing – original draft, Visualization, Resources. **Jiri Holatko:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in this study are available on request from the corresponding author.

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Appendix A. Supplementary data

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Effect of biodegradable poly-3-hydroxybutyrate amendment on the soil biochemical properties and fertility under varying sand loads

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Abstract

Background: Poly-3-hydroxybutyrate (P3HB) is a bacterial intracellular carbon and energy storage polymer, used as a thermoplastic polyester in a wide array of industrial and agricultural applications. However, how the soil microbiome and fertility are altered by exogenously applied P3HB has been relatively unexplored. This study aimed to assess the effects of P3HB addition to nutrient restricted soil: its biological properties and lettuce (*Lactuca sativa* L. var. *capitata* L.) biomass production. The experiment was designed to evaluate impacts of spatial arrangement of the relatively organic-rich (soil organic matter, P3HB particles) versus poor fractions of the matrix with confounding factors such as variable microbial biomass, inherent nutrient/energy status, different water relations (due to variable hydrophysical properties of soil augmented by sand at different ratios).

Results: The results revealed that P3HB in soils induced inconsistent to contradictory changes in the microbial abundance as well as in most enzymatic activities. The differences were conditioned by the sand content both under P3HB presence or absence. On the other hand, dehydrogenase, urease activities, basal and substrate-induced soil respirations were mostly enhanced by P3HB addition, directly with increasing sand content (several respiration types). Nevertheless, P3HB significantly inhibited lettuce biomass production.

Conclusions: P3HB introduction to soil boosts the microbial activity owing to the preferential utilization of P3HB as C source, which depletes soil N and strongly inhibits the plant growth. Enhanced microbial activity in P3HB-amended soils with high sand content (60–80%) suggested that in nutrient-impoverished soil P3HB can temporarily replace SOM as a C source for microbial communities due to the shift of their structure to preferentially P3HB-degrading microbiome.

Keywords: Soil quality deterioration, Microbial community, Respiration, Biodegradation, Lettuce, P3HB

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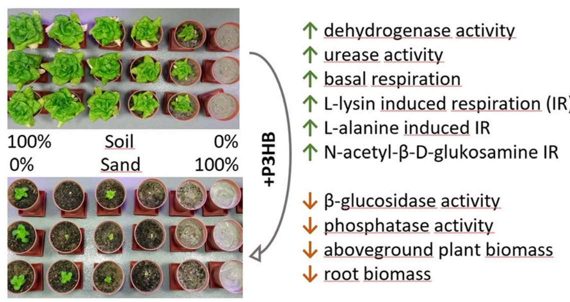
Graphical Abstract



Background

The non-degradable polymers derived from fossil fuels are in routine use worldwide. In face of the climate change and its environmental implications, the use of biodegradable polymers has been advocated recently [1, 2]. This has resulted in the increased production of various biodegradable polymers, such as polyhydroxyalkanoates (PHAs) and the most common type, poly-3-hydroxybutyrate (P3HB, for purpose of this publication), with presumably little or no negative effects on the environment. P3HB is a bacterial intracellular C and energy storage polymer, belonging to a family of biopolyesters polyhydroxyalkanoates (PHAs) [3]. PHAs are characterized by technologically promising properties such as biodegradability, biocompatibility, thermoprocessibility and flexible strengths [4]. PHB depolymerase enzymes are responsible for the biopolymer degradation [5] to monomer 3-hydroxybutyric acid which is completely microbially utilized [6, 7]. PHB has been further characterized as biodegradable under both aerobic and anaerobic conditions [8, 9], which adds a further plus to its utilization in a wide range of applications.

Given their advantages, biodegradable polymers are commonly used in agriculture [2, 10] as cover films (mulching), bands of sowing, in pots and containers and other horticulture materials and tools, for the controlled release of agricultural chemicals [11] and fertilizers [12, 13]. Owing to the wider use in daily products, other sources are similar in case of conventional plastics (i.e., compost, sewage sludge, irrigation, street runoff, littering and atmospheric deposition) [12–14]. By their degradation, the bioplastic particles are released into the soil, where their non-toxic character is assumed. However, up to now, the information on the influence of the PHB on soil quality and plant growth is scarce which demands further studies.

Zhou et al. [15] studied soil microbial community structure, growth, and exoenzyme kinetics in hotspots formed around microplastics of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). They observed that the addition of PHVB increased microbial activity and enriched specific bacterial taxa. Similar results have been observed by Deroiné et al. [16] in marine aquatic and sand environments. On the contrary, Sang et al. [17] observed reduced microbial activity in both environments.

So far, only few studies have focused on the effect of PHB amendment to soil on plant growth. The studies reported harmlessness of PHBV to maize (*Zea mays* L.) [18]. However, Mierziak et al. [19] found that an increase in the content of degradation product of PHB such 3-hydroxybutyrate (3-HB), in transgenic flax (*Linum usitatissimum* L. cv. NIKE) or after control plants treatment with 3-HB resulted in upregulation of genes involved in chromatin remodeling and activation of DNA de-/methylation. These changes were targeted to structural genes such as the phenylpropanoid pathway. Phenylpropanoid biosynthetic pathway is activated under abiotic stress conditions as the phenolic compounds attenuate the harmful impact of reactive oxygen species [20]. For example, drought stress in maize is accompanied by accumulation of phenylpropanoid [21]. Therefore, it may be considered an impact of excessive 3-HB in the soil environment on the plant ability to respond abiotic stress. In transgenic plant *C. sativa* designed to produce PHB in the plastids of seeds, high (up to 15% of mature seed weight) PHB production had varying effects on germination, emergence, and survival of seedlings; however, the survival was predominantly reduced [22]. Such finding also indicates the potential adverse impact of higher PHB levels in plants.

Under favorable conditions, the biodegradable plastic degrades fast within weeks to months [23]. These conditions occur mainly in favorable areas having soils with higher content of soil organic matter (SOM) and nutrients, higher microbial activity and C turnover, and water saturation. However, in regard to current spreadout of soil degradation and SOM deficiency in soils, plastic particles may promote undesirable soil aggregation, which reduces the accessibility of organic matter to soil microbes [24], changes the proportion of soil nutrients [25], and subsequently could decrease plant primary production due to negative influencing of soil food web [26]. As a result, soil quality and productivity decreases, which includes a decrease in microbiological activity, deceleration of nutrient turnover and a decrease in SOM content [27]. To continue the biodegradation of bioplastics, which are composed mostly of C, H and O, the degrading organisms presumably exploit other sources of nutrients in soils including those normally used by other biota including plants. The scenario of bioplastics degradation under changing conditions of soil is hard to predict or model and only partial information on these processes are currently available, especially the soil textural differences for plastics biodegradation should be further considered.

Therefore, the aim of this study was to test the influence of introduction of P3HB into the arable soil and its effects on the soil quality and fertility at different levels of sand load. Increasing sand content represents a trajectory of soil degradation—a decrease in SOM, nutrient content, and different hydrophysical conditions represent a model of declining soil quality. Therefore, using sand, the number of biodegrading microorganisms and fungi in the arable soil was diluted and the conditions for biodegradation were changed. The study aims to answer the following questions: (a) How and to what extent soil fertility and microbial communities are affected by the P3HB amendment? (b) Does the dilution influence soil fertility and microorganisms' activity proportionally? (c) How will the increasing sand proportion influence the activity of the microorganisms after P3HB amendment?

Materials and methods

Experiment design

The growth substrates used for the pot experiment were prepared by mixing a fine quartz sand (0.1–1.0 mm; $\geq$95% SiO_2) with an arable soil. The soil was a silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification) sampled (0–15 cm) near the town Troubsko, Czech Republic (49°10′28″N 16°29′32″E). The soil properties were as follows: soil macronutrients ($g \cdot kg^{-1}$)—total C 14.0, total N 1.60, P 0.097, S 0.145, Ca 3.26, Mg 0.236, K 0.231; N forms ($mg \cdot kg^{-1}$)—$N_{mineral}$ 62.8, N-NO_3 56.8, N-NH_4 6.04; pH ($CaCl_2$) 7.3. Poly-3-hydroxybutyrate (P3HB) material ENMAT Y3000 (particles < 63 μm) in the form of microparticles was obtained from TianAn Biologic Materials Co., Ltd. (Ningbo City, China). The particles had spherical or spherical-like shapes. The contact angle of P3HB was reported between 70° and ~81°, which makes it slightly hydrophobic. Further specification was reported in Fojt et al. [28].

To remove the coarse particles, the soil was sieved through a sieve with mesh size 2 mm. The sieved soil was mixed with the sand in the following weight ratios: (I) 100% soil; (II) 80% soil + 20% sand; (III) 60% soil + 40% sand; (IV) 40% soil + 60% sand; (V) 20% soil + 80% sand; (VI) 100% sand. Each treatment was prepared in two scenarios: (A) with 1 wt% P3HB; (B) without any amendment (control). The content of 1 wt% P3HB was chosen in according to results of previous experiment with P3HB [28]. In total, 6 treatments were prepared. One kg of each thoroughly mixed growth substrate type was used to fill experimental plastic pots (volume 1 L, top diameter 11 cm, bottom diameter 9 cm, height 13 cm). Each treatment was carried out in 3 replicates (pots).

The pot experiment with crop lettuce (*Lactuca sativa* L. var. *capitata* L.) cv. Brilliant took place according to the following controlled conditions: cultivation in growth chamber Climacell EVO (BMT, Czech Republic)—full-spectrum LED lighting, intensity 20 000 lx; photoperiod 12 h; temperature 18/22 °C (night/day); relative humidity 70%. A 2-day sprouting of the lettuce seeds on wet filter paper preceded sowing to the depth of approximately 2 mm in each pot. After sowing, each pot was watered with 100 mL of distilled water. The 10-day-old seedlings were reduced to only one plant (the most robust) per pot. Pot placement in the growth chamber was randomized. Manual watering of each pot with 50 mL of distilled water was done every other day. Soil humidity was controlled, and water content was maintained during the experiment. The pots were variably rotated once per week. The plants were harvested 8 weeks after sowing.

The lettuce shoots were cut at ground level, and the roots were gently cleaned of soil and washed with water. The lettuce shoots and roots were dried at 60 °C to a constant weight, and dry aboveground and root biomass were estimated gravimetrically by weighing on the analytical scales.

Soil analysis

A mixed soil sample was taken from each pot after harvesting the lettuce. Soil samples were homogenized by sieving through a sieve with mesh size 2 mm. Air dried samples were analyzed for pH [29]. Freeze-dried samples were used for the analyses of enzymatic activities: β-glucosidase (GLU), arylsulfatase (ARS), phosphatase (Phos), urease (Ure) and N-acetyl-β-D-glucosaminidase (NAG) [30]. The *p*-nitrophenol (PNP)-derivatives of the specific soil substrates were used for Vis spectrophotometric measurement (Infinite M Nano, Tecan Trading AG, Switzerland) at $\lambda = 405$ nm (β-glucosidase, arylsulfatase, phosphatase, and N-acetyl-β-D-glucosaminidase). Urease activity was determined as an amount of ammonium produced from the substrate urea, detected Vis spectrophotometrically by the reagent cyanurate ($\lambda = 650$ nm). Each soil sample was measured in nine replicates. The samples stored at 4 °C were used for determination of dehydrogenase activity (DHA) [31], soil basal respiration (BR) and substrate-induced respirations (IR). DHA was measured by 2,3,5-triphenyltetrazolium chloride (TTC)-based method. Respiration types—BR and induction with D-glucose (Glc-IR), D-trehalose (Tre-IR), N-acetyl-β-D-glucosamine (NAG-IR), L-alanine (Ala-IR), L-lysine (Lys-IR) and L-arginine (Arg-IR)—were measured using MicroResp® device (The James Hutton Institute, Scotland) and spectrophotometer (Infinite M Nano, Tecan Trading AG, Switzerland) [32].

DNA Extraction and Real-Time qPCR: DNA was extracted from 0.5 g of freeze-dried soil sample using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA). Isolated DNA was quantified using Nanodrop One (Thermo Scientific, USA). The SYBR-Green platform was used on a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, USA). Real-time PCR was performed to quantify partial bacterial (16S rDNA) and fungal (18S rDNA) genes coding for ribosomal RNA, and gene *phaZ* (coding for polyhydroxybutyrate depolymerase) in soil DNA extracts. The primers used were 1108F (5′ ATGGYTGTCGTCAGCTCGTG 3′) and 1132R (5′ GGGTTGCGCTCGTTGC 3′) for bacteria [33], FF390 (5′ AICCATTCAATCGGTAIT 3′) and FR1 (5′ CGATAACGAACGAGACCT 3′) for fungi [34], PHBf (5′ CGTCTACCGCAACGGCACCAAGG 3′) and PHBr (5′ TGGGCGTAGTTGCTGGCCGT 3′) for *phaZ* [35].

Statistical analysis

Data processing and statistical analyses were performed using freely available software R, version 3.6.1. [36]. For characterization the relationship between the treatments and selected soil properties was used principal component analysis (PCA), multivariate analysis of variance (MANOVA at 0.1% significance level) and one-way analysis of variance (ANOVA) type I (sequential) sum of squares at 5% significance level [37]. For detection the statistically significant difference among factor level means, it was used Tukey's HSD (honestly significant difference) test and "treatment contrast" to calculate factor level means for each treatment. The results were also graphically presented with Rohlf biplot for standardized PCA. Pearson correlation analysis was performed for measuring the linear dependence between soil properties. Pearson correlation coefficient was interpreted as follows: $0.0 < r < 0.3$ (negligible correlation), $0.3 < r < 0.5$ (low correlation), $0.5 < r < 0.7$ (moderate correlation), $0.7 < r < 0.9$ (high correlation), and $0.9 < r < 1.0$ (very high correlation) [38].

Results

Soil microbial communities

As expected, the effect of P3HB on 16S rDNA was variable depending on the sand content (Fig. 1A). At 0–40% of sand, the P3HB effect was neutral to negative, while at 60–100% sand content, the effect was neutral to positive. In control, the effect of soil dilution by sand on 16S rDNA was significant from 60% of sand. The similar patterns

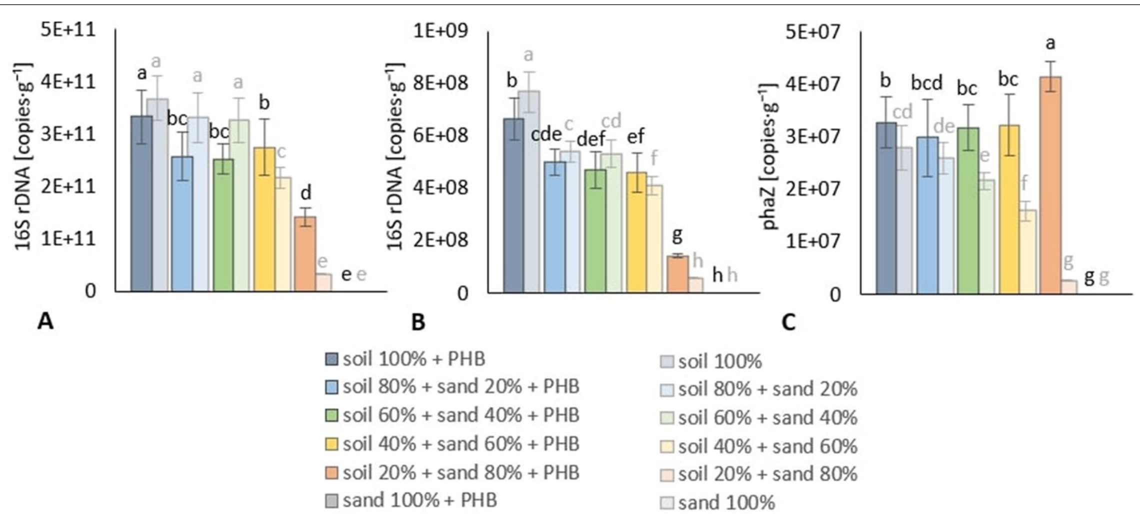


Fig. 1 **A** Bacterial (16S rDNA), **B** fungal (18S rDNA), **C** PHB-degrading microorganisms (*phaZ*) gene markers in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

were found for 18S rDNA (Fig. 1B), however the neutral-to-negative effect of P3HB prevailed until 60% of sand. In control, the effect of sand was more significant as already 20% of sand significantly reduced 18S rDNA. Presence of P3HB had neutral-to-positive effect on PHB-degrading microorganisms in all growth substrates (Fig. 1C). Furthermore, presence of P3HB alleviated the negative effect of increasing sand content (up to 80%).

Soil enzyme activities

DHA was significantly higher in P3HB-amended variants (Fig. 2A). The only exception was 100% sand variant with barely detectable DHA regardless of P3HB presence. The increasing proportion of sand predominantly led to a decrease in DHA despite its moderation to stimulation by P3HB between 60 and 80% of sand. The effect of P3HB on ARS was ambiguous depending on the sand content (Fig. 2B). At 0–40% of sand, the P3HB effect was neutral to negative, while at 60–100% of sand, the effect was neutral to positive. In the case of NAG, the P3HB effect was not clear as well, however, it was rather positive as four P3HB-amended variants had significantly increased NAG values (Fig. 2C).

P3HB increased Ure in all the variants (Fig. 2D). Decrease in Ure values related to increasing sand–soil ratio was noticeable in controls; less steep but still decreasing trend in Ure values indirectly related to raising sand content was found in the P3HB-amended variants. P3HB-amendment decreased GLU at 0–60% of sand (Fig. 2E). The effect of P3HB on Phos was differentiated depending on the sand content (Fig. 2F). At 0–40% of sand, the P3HB effect was neutral to negative, at 60–100% of sand, the effect was positive. In general, the increasing proportion of sand caused a significant gradual decrease in ARS, NAG, GLU and Phos (Fig. 2).

Soil respiration

P3HB amendment significantly increased BR at 0–80% of sand (Fig. 3A). In addition, BR increased with increasing sand content (up to 80%) in the presence of P3HB. Except

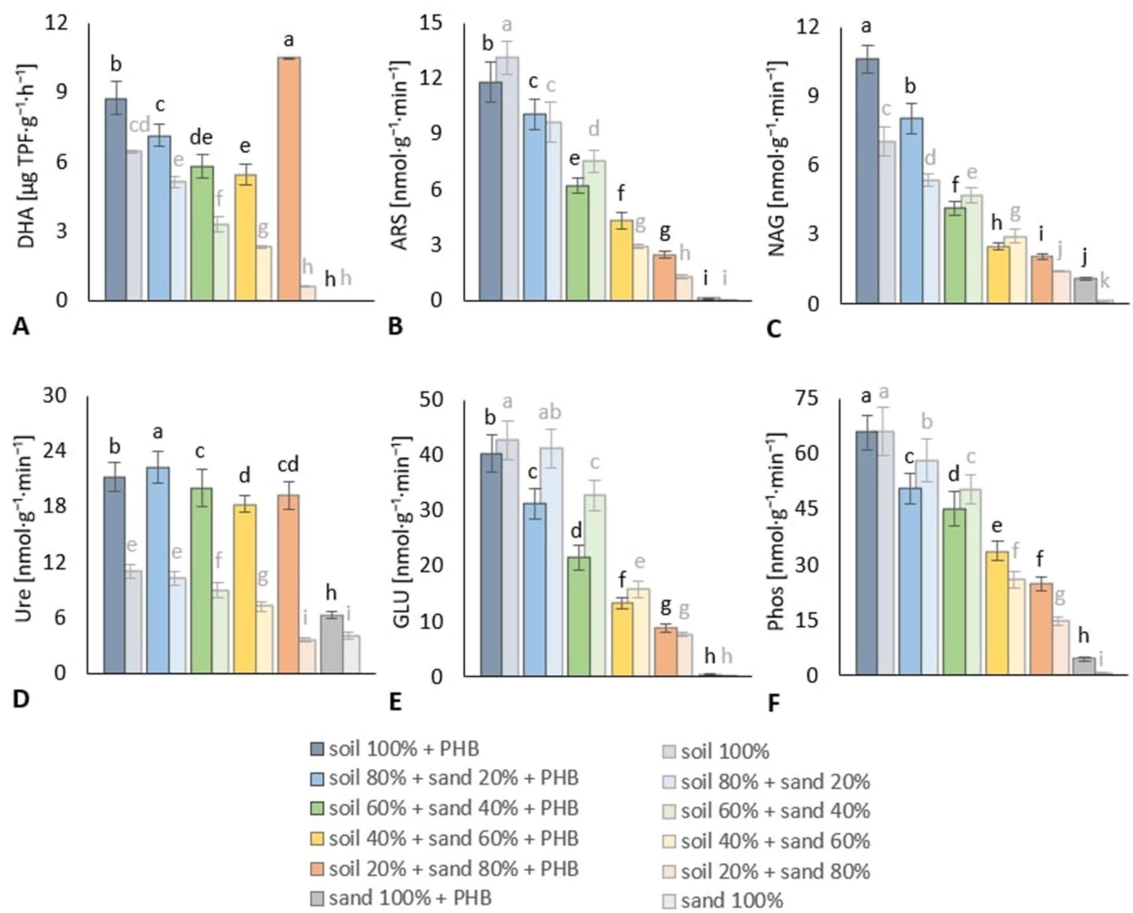


Fig. 2 Soil activities of **A** dehydrogenase activity, **B** arylsulfatase, **C** N-acetyl-β-D-glucosaminidase, **D** urease, **E** β-glucosidase and **F** phosphatase in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

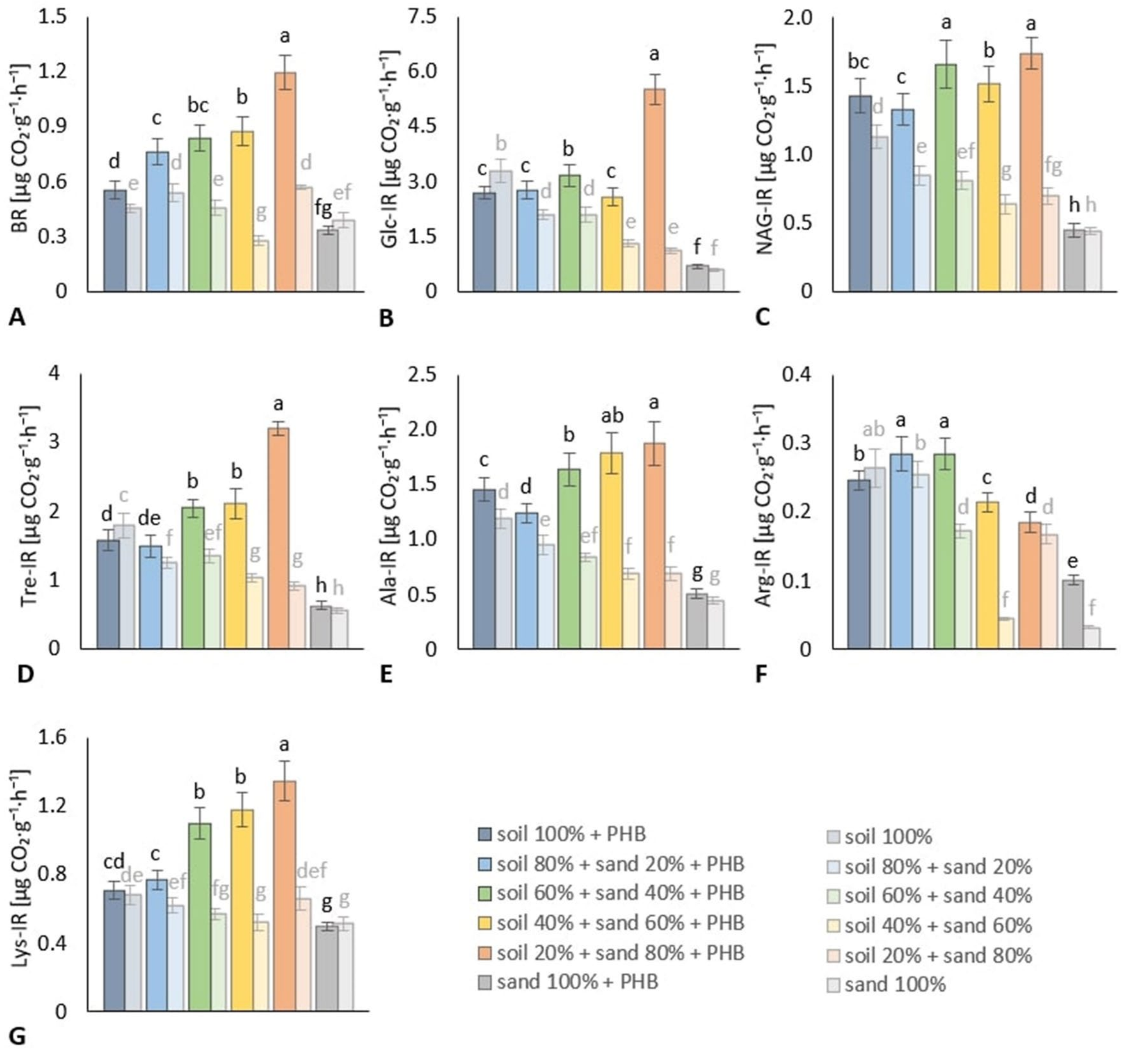


Fig. 3 **A** Basal, **B** D-glucose, **C** N-acetyl-β-D-glucosamine, **D** D-trehalose, **E** L-alanine, **F** L-arginine and **G** L-lysine induced respiration in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

for pure soil and sand variants, P3HB had a positive effect on Glc-IR (Fig. 3B). In contrast to the control, the presence of P3HB stabilized or even increased Glc-IR with increasing sand content up to 80%.

P3HB significantly increased NAG-IR in all variants containing soil (Fig. 3C). In addition, the negative effect of increasing sand content on NAG-IR in the control contrasted with P3HB-amended variants up to a sand level of 80%. The same trend was visible in Tre-IR (Fig. 3D) as both respirations highly correlated (Additional file 1: Fig. S1). P3HB effect was positive as well, except pure soil and sand variants. The results' trends of Ala-IR and Lys-IR (Fig. 3E, G) followed the results of Glc-IR and Tre-IR as is also reflected by their positive significant correlations (Additional file 1: Fig. S1).

Arg-IR slightly differed compared to other respirations (Fig. 3F). The effect of P3HB was neutral to positive as well, however, the respiration increase with increasing sand content in the presence of P3HB was not recorded. Although there was a significant increase in Arg-IR at 20% of sand, further sand content increase was followed by unchanged or decreasing Arg-IR.

Plant biomass

Fresh and dry aboveground plant biomass (Fig. 4A, B) as well as dry plant root biomass (Fig. 4C) were seriously negatively affected by P3HB as all soil containing variants showed significantly lower values compared to the control. The only exception was 100% sand variant, which had similar negative effect on plant biomass production

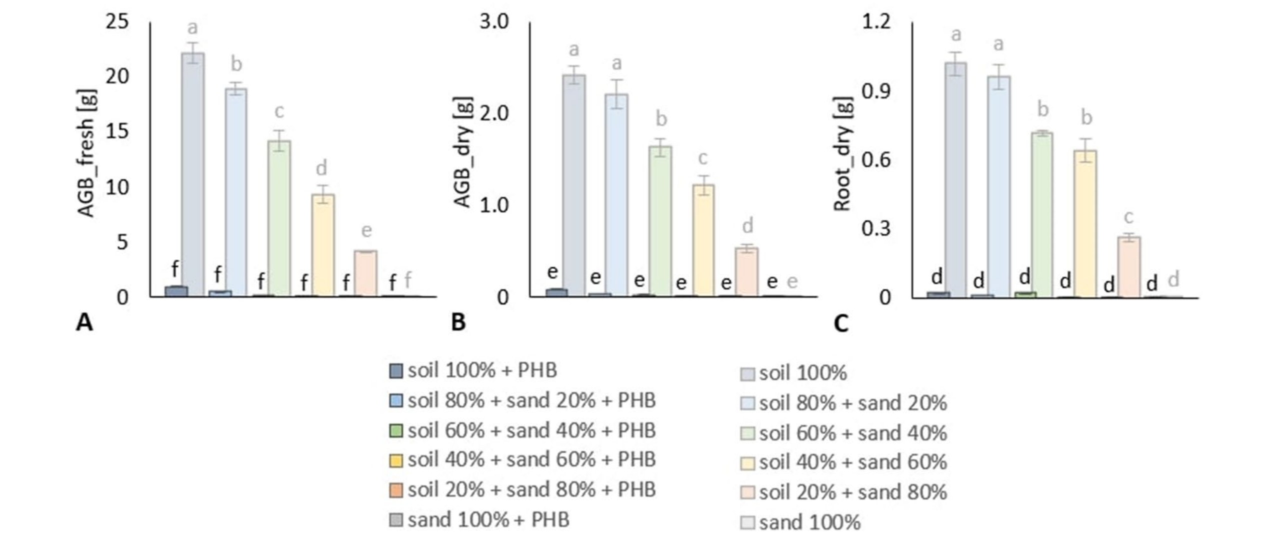


Fig. 4 **A** Fresh and **B** dry aboveground plant biomass (AGB) and **C** dry root plant biomass in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

regardless of P3HB presence. In control, decreasing plant biomass production followed increasing sand content; in P3HB-amended growth substrates, this trend was suppressed.

Discussion

Effect of P3HB on soil microbial activity

The microbial behavior after addition of P3HB was similar as after PHBV addition [15] with specifics caused by dilution of soil or SOM by increasing sand content. The effect of P3HB on 16S rDNA and 18S rDNA was ambiguous, as P3HB addition reduced, increased, or did not affect bacterial and fungal content (Fig. 1A, B). The results suggest that the mechanism of their interaction and resulting effect may be related to changing physical and chemical properties of soil (see further discussion). PHB-degrading microorganisms (Fig. 1C), on the other hand, were predominantly positively affected by P3HB addition to soil. This increase is probably directly related to the supply of the preferred energy source in the form of a biodegradable polymer [5, 39]. Due to the suppression of the influence of P3HB in the sandy (100%) substrate due to the assumed negligible content of SOM and microbiota (Fig. 1), we do not discuss this variant in this chapter.

The individual enzymatic activities shed light on the processes occurring in soil after addition of P3HB. Ure is an important extracellular enzyme that hydrolyzes urea and regulates the early nitrification process in soil and is closely related to the SOM content [40]. Therefore, it is related to the N availability. Ure is the only enzyme that showed disproportional decrease with increasing sand content (Fig. 2) as in soils without P3HB. This indicates that the P3HB increases activity and abundance of nitrifying microbes which cause enhanced Ure activity independently of SOM content. Thus, P3HB can replace (temporarily) the SOM in sandy soil.

The P3HB is composed of C, O and H, therefore the immobilization of essential nutrients (mainly N and P) is necessary for microbial degradation [15, 41]. This comes from the notion that an optimal C:N ratio in soil is around 25.0 [42]; above this value N is immobilized and below this value is N mineralized [43]. In this work, P3HB application increased soil C:N ratio. During biodegradation, this increase caused N shortage around PHB particles, and the microbes immobilize N from their environment [44]. This generally results in a decrease in plant-available N in soil. If there is no N to immobilize, microbial growth is slowed down. By addition of P3HB is the C:N disturbed, while the disruption seems to be the most pronounced in samples with low content of SOM. Noteworthy, the imbalance in C:N ratio influences the soil organisms in a different way; fungi have wider C:N ratios in their tissues than bacteria and archaea and therefore, they can grow more efficiently on low N substrates and will thus mineralize N more readily. This is also the case of PHB, which is biodegraded preferably by fungal communities at the phylum level dominated by *Ascomycota* [45]. The product of urea hydrolysis is CO_2 and NH_4^+; the latter is either used by plants or microbes, which can eventually convert it into NO_3- via nitrification processes

[46] However, nitrification rates are typically low as the nitrifiers are relatively poor competitors for NH_4^+ in the soil solution and occurs when the NH_4^+ supply exceeds plant and other heterotrophs demand [47]. As the plant growth was largely suppressed under P3HB addition (Fig. 4), it can be anticipated that majority of NH_4^+ was used for growth of soil microbes, especially of PHB-degrading microorganisms (Fig. 1C). This enhanced consumption of ammonium nitrogen putatively more decreased the content of plant available, inorganic nitrogen, similarly as referred to polylactic acid (PLA) contaminated soil during vegetative state of bean [48]. This hypothesis is supported by the enhanced Ure activity (Fig. 2D) and high correlation of Ure activity with the total degradation level indicated by DHA and PHB-degrading microorganisms (Additional file 1: Fig. S1). In addition, Zhou et al. [15] reported an increased activity of *Acidobacteria* and *Verrucomicrobia* phyla in soils degrading PHBV. In fact, neither *Acidobacteriota* [49] nor *Verrucomicrobia* [50] are involved in soil N-cycle processes such as nitrification, denitrification, or nitrogen fixation. Thus, it can be concluded that N (or its vast majority) is used for growing of microorganisms' population.

This finding confirms the earlier assumption of Hoshino et al. [51] who explained better correlation of the biodegradable polymers degradation with the total N content than with the total C content by the necessity of soil N for the degradation by microorganisms as it is absent in bioplastics.

Importantly, unlike the Ure the phosphatase activity showed proportional results in terms of sand content (Fig. 2F). Phosphatase is an extracellular enzyme that mineralizes organic P into phosphate by hydrolyzing phosphoric (mono) ester bonds [52]. Similarly to all extracellular phosphatases, enzyme expression is induced by P deficiency [53]. Notably, Fig. 2F shows higher demand for P in soils containing P3HB and high sand content comparing to soils with lower sand content.

DHA is a basic indicator of microbial activities coupled with SOM degradation in soil [54]. The most important function of DHA is the biological oxidation of SOM, achieved by transferring protons and electrons from organic substrates to inorganic acceptors [55]. For this reason, DHA positively correlates with SOC and C_{org} as it reflects the activity of living cells and not of DHA stabilized in soil complexes [53]. This explains its positive correlation with other microbe-related soil properties (Additional file 1: Fig. S1). In addition, significantly increased DHA values in P3HB-amended variants (Fig. 2A) assumed that PHB addition enhanced soil degradation rate due to its utilization as an energy/C source [15, 56, 57]. This assumption was confirmed especially by the prevailing increase in PHB-degrading microorganisms (Fig. 1C) and their high positive correlation with DHA (Additional file 1: Fig. S1).

ARS is involved in the S mineralization process which cleaves organosulphates [58] and is used as a measure of soil health and soil microbial activity [59]. ARS activity is correlated with soil microbial biomass and the rate of S immobilization [60], pH and SOC [61]. Here, similarly as phosphatase, its activity is elevated in sandy soils (> 60% sand) with P3HB (Fig. 2B), which reflects higher demand for immobilization of S in less buffered system.

NAG is an enzyme catalyzing the hydrolysis of terminal 1,4 linked N-acetyl-beta-D-glucosaminide residues in chitooligosaccharides, i.e., it is involved in degradation of chitin, the key polysaccharide of fungal cell wall [62]. Its activity was enhanced in most P3HB-amended variants (Fig. 2C), which underlines higher demand of degrading organisms for N acquisition from available sources, but different than Ure, as suggested by their low correlation (Additional file 1: Fig. S1). Enhanced NAG activity supports the view that plants or microbes may use N-containing monomers and not only inorganic N [63].

GLU is an enzyme that catalyzes the hydrolysis of terminal 1,4 linked β-D-glucose residues from β-D-glucosides, including cellulose oligomers and thus it is an indicator of SOM degradation and soil C utilization [64]. Its activity was either the same or even lower in soils with P3HB (Fig. 2E). This may be attributed to either preferable cleavage of PHB rather than cellulose or a high C:N ratio [64].

BR is a key indicator of aerobic catabolic activity in soil, and accessibility and degradability of organic C in SOM [65]. The P3HB application positively influenced BR (Fig. 3A) according to the mechanism described above in the DHA-related part. Substrate-induced respiration is used to measure the activity of specific microorganisms responding to addition of substrates with specific composition. Response induced by Glc, NAG, Tre, Ala and Lys corroborated with results of BR (Fig. 3). Similar mechanisms and involvement of the same organisms is a probable reason of the grouping of all these respirations (Additional file 1: Fig. S2) and their positive significant correlation (Additional file 1: Fig. S1). On the contrary, arginine substrate showed slightly different results comparing to them.

The results of Arg.IR, an indicator of fungal respiratory activity in soil, suggest neutral-to-positive effect of P3HB as well (Fig. 3F). However, a slight deviation in the trend of values from the results of other respirations is visible (Fig. 3) and evident also from the PCA (Additional file 1: Fig. S2). The respiration decreases with increasing sand content. This indicates the relation of Arg-IR to soil texture. In sandy soils, the pores are better aerated, water content and water holding capacity are significantly reduced. These conditions probably limited the use of arginine substrate by microorganisms.

Effect of P3HB on soil fertility

The results clearly indicate an adverse effect of P3HB on both above- and below-ground biomass production of *L. sativa* (Fig. 4). These results are in accordance with previous works that degradation of biodegradable plastics might negatively affect plant growth [66, 67]. In general, for biodegradation of P3HB, two major explanations may be attributed to the observed effects: (i) phytotoxicity of P3HB microplastics or their degradation products and (ii) the effect on soil properties and/or inhibition of nutrients.

Alternative (i) was thoroughly discussed by Zhou et al. [15] who observed similar results as in this work by testing PHBV, which is a common derivative of PHB. The authors speculated about possible phytotoxic effect of PHB biodegradation products due to acidification of soil caused by released of 3-hydroxybutyric acid during PHBV degradation, but this speculation was in the cited work rejected. The rejection of the hypothesis is partially in line with our results showing the resulting pH above 7 in soil without P3HB (in other soils was probably increased due to sand content). Moreover, the pKa of 3-hydroxybutyric acid is 4.41 [68] indicating that the acidification effect of this acid is very low. Influence of 3-HB effect on the phenylpropanoid pathway regulation related to reaction on abiotic stress [19, 20] was also mentioned. Liwarska-Bizukojc [69] used *S. saccharatum, S. alba* and *L. sativum* as phytotoxicity bioindicators of PHB and found no effect on seed germination even at concentration as high as 11.9% w/w. Nevertheless, the presence of PHB in soil caused root growth inhibition mainly on *Sinapsis alba* and *Lepidium sativum*. On the contrary, Dahal et al. [18] did not find any significant effect of PHBV on plant growth. Possible adverse effect of PHB due to reduced seedlings survival was indirectly suggested by [21].

Alternative ii) was discussed by Silveira Alves et al. [70] who stated that the role of PHB in plant-bacterium interactions is still poorly understood, however, their study suggested that PHB metabolism may contribute to bacterial plant growth promotion and that deletion of genes involved in the synthesis and degradation of PHB reduce the bacterial ability to enhance plant growth [70]. Here, despite the prevailed P3HB-related promotion of microorganisms' abundance and community structure, the suggestion of plant growth promotion must be rejected. Furthermore, due to the hydrophobic nature of PHB [71], a higher water repellency and increased drain-off may be expected in PHB-amended substrates. Zhou et al. [15] concluded that PHBV addition increased microbial activity, growth, and exoenzyme activity, changed the soil bacterial community at different taxonomical levels and increased the alpha diversity, which most likely led to the enhanced mineralization of native SOM and negatively influenced the growth of *Triticum aestivum* L.

Our results, i.e., serious P3HB-related growth inhibition of *L. sativa* (Fig. 4) and enhanced microbial activity, are supported by the results reported in [15]. As indicated by the low negative correlations between plant biomass and BR, Lys-IR and Ure, respectively (Additional file 1: Fig. S1), growth inhibition could result from an adverse consequence of plant-microbiota interaction, such as competition for nutrients. The most likely scenario appears to be competition for N, which was probably utilized by PHB-degrading microorganisms (as discussed above). Similarly, suppressed growth of common bean shoots and roots in PLA-treated sandy soil, reported by [48], was likely caused by significant deficit of plant available (mainly nitrate nitrogen) and disproportion in dissolved organic carbon (DOC) and nitrogen (DON), leading to increased C:N ratio. Noteworthy, in our case, we can exclude the negative effect of drought stress caused by hydrophobicity of PHB as the design of pot experiments (i.e., regular irrigation) exclude the possibility of shortage of moisture in soils due to regular irrigation.

Effect of P3HB under changing SOM content as an implication of soil degradation

The results of multivariate analysis of variance (MANOVA) showed significant ($p<0.001$) differences among experimental variants in all determined properties confirming the importance of increasing sand content in arable soils for the use of P3HB in agricultural soils. However, the consequences were variable, as they were positive, neutral, and negative.

16S rDNA and 18S rDNA (Fig. 1A, B) were very highly positively correlating (Additional file 1: Fig. S1) and followed similar pattern of overall decrease in both P3HB-amended and control substrates. Despite clear disproportionality, the decrease was probably caused by the decrease of the SOM content and number of soil microorganisms and fungi following soil dilution by sand. This also negatively affected all enzymatic activities, which showed a similar overall trend (Fig. 2). Therefore, the progressive deterioration of soil quality by the increase in the sand fraction will have a negative impact on microbial and fungal biomass and enzymatic activities, whether the soil is contaminated with P3HB or not.

However, the degree of sand influence is significantly affected by the presence of P3HB. Compared to control, bacterial and fungal biomass content (Fig. 1A, B) followed similar pattern of initial stagnation or decrease after P3HB addition replaced by stagnation or growth at higher ($\geq 60\%$) sand load. In PHB-degrading microbiome, the negative effect of increasing sand content was alleviated by presence of P3HB (Fig. 1C). Moreover, the results suggest that presence of P3HB together with increasing sand content (up to 80% of sand) can even stimulate PHB-degrading microorganisms. Thus, the partial increase of 16S rDNA and 18S rDNA was probably related to this part of the microbiome. The reason may be better aeration of the substrate accompanied by the necessary presence of the P3HB energy source substituting declining SOM content, resulting in the booming of PHB-degrading microorganisms and overall shift in microbiome structure. This hypothesis is supported especially by the similar phenomenon in DHA (Fig. 2A), increasing respirations with increasing sand content (Fig. 3) and the grouping of these factors in the PCA (Additional file 1: Fig. S2).

Some other enzymes (ARS, NAG, Ure, GLU, Phos) also showed partial deviations indicating a different effect of P3HB depending on the sand content (Fig. 2). For example, at lower sand content (0–40%), the effect of P3HB on ARS (Fig. 2B) and Phos (Fig. 2F) was neutral to negative, while at higher sand content (60–100%), the P3HB effect was neutral to positive. This suggests that the expected PHB-degrading microorganisms boom related to the increased aeration had a positive effect on these enzymatic activities as well. Therefore, although P3HB acts as a potential selective microbial inhibitor in a favorable state of soil due to the dominance of different (natural) functional groups of microorganisms, in unfavorable conditions of increasing sand content, P3HB can maintain or even stimulate the activity of some enzymes associated especially with the PHB-specific microbes as an alternative energy source.

As already mentioned, predominant stimulation of BR, Glc-IR, NAG-IR, Tre-IR, Ala-IR and Lys-IR with increasing (up to 80%) sand content in P3HB-amended substrates (Fig. 3) was probably caused by better aeration coupled with P3HB utilization. This phenomenon could also be explained by the increasing rate of preferable utilization of P3HB with the increasing portion of PHB-derived C regarding the total soil organic C. This feature might be comparable to the observation of Kuzyakov and Bol [72], who described a metabolism switch from the hardly utilizable recalcitrant C in SOM to the easily available carbonaceous compound leading a positive priming effect. The study carried out with bean-planted PLA-contaminated sandy soil also showed increasing values of readily oxidizable carbon (POXC) with ascending content of plastics (up to 2%) [48]. However, this can be considered as a necessary side effect rather than a cause, as it would have a similar graded positive effect on the other characteristics studied.

The increase in sand content significantly reduced the production of plant biomass in the control (Fig. 4), however, the presence of P3HB suppressed this phenomenon and limited biomass production to a minimum. Therefore, the gradual degradation of P3HB-contaminated arable soils by increasing sand content is not determining for *L. sativa* yields; P3HB presence limits the growth of *L. sativa* to the same extent as growing in pure sand. It is important to keep in mind that the possible contamination of soil by 1% of P3HB is realistic in the case of application PHB-based fertilizer coatings, delivery systems and mulching films. The SOC content in the soil was 14 g kg^{-1}, therefore, applied dose of P3HB is very high in terms of SOM-to-P3HB ratio. As the P3HB is easily available substrate, we speculate that even significantly lower dose may have an adverse effect on plant growth. A decrease in SOM (represented here by sand dilution) can worsen this adverse effect .

In summary, the effect of changing sand content is also well reflected in the PCA (Additional file 1: Fig. S2). Sand's dominant property, high pH (Additional file 1: Fig. S3), is clearly related to the sandy variants, which form one separate group of substrates unsuitable for soil organisms and plant biomass production. The absence of P3HB and high soil content are clearly the most important factors for high *L. sativa* biomass production. High soil content is key for high level of bacterial and fungal biomass, as well as most enzymatic activities. The presence of P3HB and a more balanced soil:sand ratio is both crucial for high soil respiration and content of PHB-degrading microorganisms.

Conclusions

The changes in the microbial abundance and community structure after P3HB addition were variable; however, there was a growth and amplification of specific PHB-degrading microbial population. The results of enzymatic activities were also ambiguous depending on the sand content. The basal and substrate-induced respirations as well as DHA were mostly enhanced by the P3HB addition, which seemed to be preferable source of C and energy for soil aerobic microbes. In conclusion, although P3HB acts as a potential selective microbial inhibitor in a favorable state of soil, in unfavorable conditions of increasing sand content (approximately 60–80%), P3HB can maintain or even stimulate the microbial abundance and community structure as well as enzyme activities and soil respiration. However, these potentially positive effects on the soil microbiome were contrasted by the significant adverse effects on the plant growth. The results show that soils may have the capacity to degrade the bioplastics, but at the cost of nutrient availability to plants and negative impact on their growth. This capacity may be affected by a gradual nutrient-related deterioration of soil quality. Moreover, at low SOM content (increased sand-to-soil ratio), the P3HB can replace the SOM as a main substrate, which shifts the composition of microbial community towards this only available substrate. We conclude that, although the biodegradable bioplastics are C neutral, further research should answer if they do not induce positive priming effect on SOM, as the overall activity of soil organisms were disproportionately boosted in P3HB-amended sandy soils. Most importantly, future studies should find the conditions under which can biodegradable plastics enter the soils without any adverse effect on soil fertility and other properties.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00345-9.

Additional file 1: Fig. S1. Correlation matrix of soil properties; numbers indicate the Pearson's correlation coefficient r. **Fig. S2.** Rohlf PCA biplot of individuals and variables. **Fig. S3.** pH in the substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

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Author contributions
Conceptualization, TH, JH and MB; methodology, TH, JH and MB; software, TH, TB, JF; validation, VP, MB, AM, OM, AK and JK; formal analysis, JF, TB; investigation, JH, AM and MB; resources, JH, TH, and AK; data curation, TH, MR and JF; writing—original draft preparation, JH, MB; writing—review and editing, VP, TH, JH, AM, JK and MB; visualization, TH and AK; supervision, JK, VP; project administration, JF, JH, MB, AK; funding acquisition, MB, AK, JH, JK. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Soil texture-driven modulation of poly-3-hydroxybutyrate (P3HB) biodegradation: Microbial shifts, and trade-offs between nutrient availability and lettuce growth

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ABSTRACT

Poly-3-hydroxybutyrate (P3HB) is a promising alternative to persistent conventional plastics, capable of biodegrading within months. However, its microbial-driven degradation raises concerns about nutrient immobilization and impacts on plant growth. The biodegradation process occurs in multiple stages, during which shifts in the microbial community can alter soil properties and influence utilization of both intrinsic and polymer-derived organic matter.

This study employs a novel approach to investigate how nutrient dynamics during the late stage of P3HB biodegradation affect lettuce (*Lactuca sativa* var. *capitata* cv. Brilliant) growth. Soil-to-sand mixtures (100_0, 80_20, 60_40, 40_60, 20_80, and 0_100 ratios) were spiked with P3HB, allowed to biodegrade for eight weeks, and then planted with sprouted lettuce seeds, which were cultivated for another eight weeks.

P3HB addition inhibited plant growth and root development in all soil-sand mixtures. However, increasing the sand proportion enhanced plants' nitrogen content by 13–45 % compared to 100 % soil + P3HB. Depending on the sand-to-soil ratio, P3HB stimulated most enzymes involved in carbon, nitrogen and phosphorus acquisition. Basal and substrate-induced respirations were 9–209 % higher under P3HB addition compared to P3HB-free soil, likely due to an increase in the stabilized soil organic matter fraction.

Residual P3HB analysis revealed that diluting soil with 20 % sand accelerated biodegradation, despite a decrease in bacterial abundance. In the 80_20 variant, the microbial community shifted toward higher fungal abundance, 19 % more than in 100_0 soil. While microbial proliferation was observed, it effect was outweighed by negative impacts on dry aboveground and root biomass. The highest P3HB biodegradation rate occurred in the 80_20 variant, underscoring soil texture as a critical factor in P3HB biodegradation. While microbial

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communities can degrade bioplastics, this process may compromise plant nutrient availability and hinder plant growth.

1. Introduction

Bioplastics, a class of polymers derived from renewable and biodegradable sources, offer a promising future for improving agricultural and environmental conservation practices. Their adoption, primarily due to their non-toxic properties, holds the potential to revolutionize farming and environmental sustainability (Luckachan and Pillai, 2011; Schöpfer et al., 2022). The increasing awareness of the consequences of plastic pollution, climate change, and other environmental issues has spurred a transition toward biodegradable polymers, offering hope for a more sustainable future (Dahman and Ugwu, 2014; Dahal et al., 2020).

Poly-3-hydroxybutyrate (P3HB) is a completely biodegradable and biocompatible polymer obtained through bacterial fermentation of renewable resources, which makes it exceptionally adaptable for various applications (Yu et al., 2006; Albuquerque et al., 2020). P3HB has a unique combination of properties that are desirable for multiple applications. Moreover, it demonstrates high resistance to chemicals, excellent water vapor barrier properties, antimicrobial qualities, and the ability to form diverse structures (Baei et al., 2011). The utilization of P3HB in agriculture is gaining popularity due to its versatility and cost-effectiveness, aligning with the increasing demand for sustainable and environmentally friendly agricultural practices (Ngo, 2020). In particular, P3HB can be used as a material for mulches (Kaisrajan and Ngouajio, 2012) and fertilizer coating (Volova et al., 2016) or delivery systems (Boyandin et al., 2016).

Despite the wide range of potential applications for P3HB, not many studies have investigated its effects on soil health and crop production yet (Brown et al., 2023; Brtnicky et al., 2024b; Serrano-Ruiz et al., 2023; Zhou et al., 2021). Moreover, while the potential benefits of using bio-based plastics in agriculture are well recognized, the potential risks associated with using biodegradable bioplastics, such as changes in soil moisture retention (Fojt et al., 2022), pH (Brtnicky et al., 2022; Zhou et al., 2021), microbial community structure (Raghuwanshi et al., 2018; Reay et al., 2024), and activity (Brtnicky et al., 2024a; Palucha et al., 2024), are often overlooked. One reason for the changes is the formation of the plastisphere, a new ecological niche consisting of microbial communities that form biofilms and colonize plastic debris (Schöpfer et al., 2022; Zhou et al., 2021). Plastisphere can shift the microbiome structure and composition, resulting in differences in metabolic pathways and functions compared to the surrounding soil, including the changes in fluxes of available nutrients and microbial activity (Hu et al., 2023; Qi et al., 2022; Zhou et al., 2021). Plastisphere of biodegradable mulch film had higher abundances of specific bacterial phyla than the bulk soil (Hu et al., 2023; Zhou et al., 2021); for example, plastisphere around biodegradable plastic blend of PBAT-PLA (polybutylene adipate terephthalate – polylactic acid) exhibited different functional and taxonomic traits related to nitrogen (N) cycling, compared to bulk soil. PBAT-PLA promoted diverse N transformations, enriching genes for N fixation, degradation, and assimilatory nitrate reduction while inhibiting nitrification and denitrification. The plastisphere of PBAT-PLA harbored high abundances of *Proteobacteria*, especially genera like *Sphingomonas* and *Ramlibacter*, which drove differences in N cycling processes; *Ramlibacter* strains, which support ammonium generation and transport, contributed to increased soil NH_4^+-N (Hu et al., 2023). The higher abundance of the N-utilizing group *Nitrospirae* following the addition of PHBV (poly-3-hydroxybutyrate-co-3-hydroxyvalerate) indicated a shift in N cycling as well (Zhou et al., 2021). These findings suggest that the degradation of biodegradable plastics enhances microbial N immobilization and soil N bioavailability, activity, and abundance of specific soil microorganisms.

The stimulation of microbial activity intensifies the immobilization of macronutrients, which may negatively impact plant growth (Reay et al., 2024; Xing et al., 2025). In our previous study (Brtnicky et al., 2022), the addition of P3HB to soil enhanced the activity of dehydrogenase, urease, and phosphatase enzymes, i.e. enzymes involved in carbon (C) utilization and cycling of nitrogen (N) and phosphorus (P). Similarly, Reay et al. (2024) reported comparable effects on C, N, and P-metabolizing enzymes in response to high PHBV doses in soil. In various soil-to-sand dilution treatments, where soil organic matter (SOM) content varied, dehydrogenase activity increased from 25 to 95 % and urease from 50 to 80 %, whereas phosphatase increased mainly at high soil-to-sand dilution ratios (from 20 to 60 %). The enhanced microbial activity decreased the biomass of cultivated lettuce (Brtnicky et al., 2022; Reay et al., 2024). On the contrary (Sang et al., 2004), reported that P3HB addition decreased microbial activity. This discrepancy in findings could be due to different experimental conditions or the specific characteristics of the soil and plant species used in the studies. Adverse effects of biodegradable plastics (BDP) (based on polysaccharide) on wheat growth and development were also reported by (Qi et al., 2018), which was attributed to the competition for nutrients between plant and soil microorganisms. P3HB micro-bioplastics increased the rate of water evaporation, thereby promoting soil desiccation similar to microplastics made from polyethylene terephthalate (Fojt et al., 2022). This indicates a potential exacerbation of BDP-derived adverse effects on plant vigor and growth. A ten-month experiment revealed that the biodegradation of P3HB without plant cultivation decreased SOM content in Phaeozem by 15 %, Cambisol by 5 %, and Chermozem by 3 % (Palucha et al., 2024). The decrease was attributed to the stimulation of microbial activity, which intensified SOM decomposition as reported by other authors (Schimel and Schaeffer, 2012).

Thus, the use of biodegradable plastics in agriculture may alter mass and energy flows in soil, affecting both quantitative (biomass) and qualitative (diversity and composition) properties of the microbiome. The impact of P3HB degradation and its byproducts on nutrient (primarily carbon) balances across different SOM pools, including C fraction of varying stability, remains a largely unexplored area. This is particularly relevant, when considering the link between P3HB-indiced changes in SOM and the observed (or anticipated) decline in plant growth and soil health. Only few studies have addressed the relationship between P3HB degradation rate and soil type (Barak et al., 1991; Boyandin et al., 2011; Palucha et al., 2024). Furthermore, apart from Palucha et al. (2024) no research has investigated how microbial decomposition of intrinsic SOM is affected by P3HB utilization as a carbon source and its connection to enhanced nutrient acquisition. With increasing socio-economic demands for bioplastics as an alternative to non-degradable plastics, their use in agriculture, whether as a fertilizer coatings or plastic films, is expected to rise in response to growing environmental concerns. Therefore, understanding the effects of P3HB on soil and plant health is crucial for the sustainable use of bioplastics in agriculture.

Previous studies on the impact of biodegradable plastics on plant growth have typically followed a similar experimental design, in which soil was contaminated with plastic simultaneously with plant cultivation. However, plastic (bio)degradation is a multistep process that includes (bio)deterioration, (bio)fragmentation, assimilation, and mineralization (Silva et al., 2023). This process is coupled with formation of a biofilm (plastisphere) (Rüthi et al., 2020)) on the plastic surface, which alters surface properties (Silva et al., 2023), influences microbial activity and ultimately impacts soil quality and plant growth, depending on the stage of degradation (Palucha et al., 2024). This is exactly the uniqueness of the presented study, as its research and experimental design evaluates the effect of soil amendment with P3HB eight weeks before plant cultivation. The study aimed to determine how changes in soil's physico-chemical and biological traits during the intermediate phase of P3HB degradation, occurring before the interaction with test plants, were influenced either by the delayed involvement of the rhizosphere in the decomposition process (similarly as reported by e. g. (Boyandin et al., 2011; Brtnicky et al., 2022) or by the delayed onset of severe nutrient limitation due to intense competitions for remaining resources between the multiplied microbiome and the plants.

This semi-controlled design models a hypothetical scenario in environmental and agricultural practice, when soil contaminated with the remains of BDP plastics (e.g. mulching films, coatings or landfill residues) undergoes greening or agricultural management at various stages of degradation. Accordingly, the specific objectives of this study were to assess the effects of P3HB addition into the soil on i) microbial respiration and enzyme activity, ii) microbial abundance and community composition, iii) plant growth, and content of N, and P and iv) to investigate the influence of the soil-to-sand dilution ratio to the rate of P3HB biodegradation. We hypothesized that I) the P3HB addition would reduce plant biomass and shift soil microbial activity and community composition towards specific bacterial phyla related to N transformation processes and P3HB biodegradation, and II) this effect would depend upon the soil-to-sand dilution levels, as increased sand content reduces SOM and microbial abundance, both of which are factors supporting the rate of biodegradation.

2. Materials and methods

2.1. Experimental design and treatments description

The soil had a silty clay loam texture (according to the USDA Textural Triangle), was sampled (0–15 cm) near the town of Troubsko, Czech Republic (49°10′28″N 16°29′32″E), and classified as a Haplic Luvisol according to the WRB. The soil properties were: macronutrients ($g \cdot kg^{-1}$): total C 14.0, total N 1.60, P 0.097, S 0.145, Ca 3.26, Mg 0.236, K 0.231; N forms ($mg \cdot kg^{-1}$): $N_{mineral}$ 62.8, N-NO_3 56.8, N-NH_4 6.04; pH ($CaCl_2$) was 7.3. The last crop before soil sampling was winter wheat, which was fertilized by local standard method, i.e. by 120 kg of nitrogen per hectare.

The growth substrates utilized in the pot experiment were prepared by mixing fine quartz sand (0.1–1.0 mm; ≥95 % SiO_2) with the soil, which was done to control the level of aeration, and to simulate the level of soil nutrients. To eliminate coarse particles, the soil was sieved through a 2 mm mesh sieve. The sieved soil was then mixed with sand in the following weight ratios: (I) 100 % soil (further in the text abbreviated as 100_0), (II) 80 % soil + 20 % sand (80_20); (III) 60 % soil + 40 % sand (60_40); (IV) 40 % soil + 60 % sand (40_60); (V) 20 % soil + 80 % sand (20_80); (VI) 100 % sand (0_100). Such a dilution changed the soil texture and resulted in: (I) silty clay loam, (II) loam, (III) loam, (IV) sandy loam, (V) loamy sand, and (VI) 100 % sand (see SI). This experimental design was chosen to simulate soil with varying texture and a decreasing content of intrinsic SOM, an approach with practical implications for real-world agricultural systems that utilize different soil types. Previous studies have reported how soil type can influence P3HB degradation (Barak et al., 1991; Boyandin et al., 2011; Palucha et al., 2024).

The substrate was then divided into two pools: (A) without any amendment (control = P3HB "No"); (B) with 1 wt% poly-3-hydroxybutyrate (P3HB "Yes") which was based on the results of a previous experiment with P3HB (Brtnicky et al., 2022). ENMAT Y3000P was used (particle size <63 μm), in the form of microparticles, which was procured from TianAn Biologic Materials Co., Ltd. (Ningbo City, China). The particles had spherical or spherical-like shapes, the contact angle of P3HB was between 70 and ~81°, rendering it slightly hydrophobic (Fojt et al., 2022). In total, 12 treatments (6 without amendment, 6 with P3HB) were prepared. One kilogram of each thoroughly mixed growth substrate type was used to fill experimental plastic pots (volume 1 L, top diameter 11 cm, bottom diameter 9 cm, height 13 cm). Each treatment was carried out in 3 replicates (pots).

2.2. Plant growth experiment

The pot experiment was organized into a completely randomized design and lasted for 8 weeks. The following conditions were controlled in a growth chamber (Climacell EVO, BMT, Czech Republic): light was full-spectrum LED at an intensity of 20,000 lx, a photoperiod of 12 h, temperatures of 18/22 °C (night/day) and a relative humidity of 70 %. Each pot was added with 50 mL of distilled water every other day, and moisture was controlled by weight. After 8 weeks, two-day-old, sprouted seeds of lettuce (*Lactuca sativa* L. var. *capitata L.*) cv. Brilliant were planted to each pot to a soil depth of approximately 2 mm. Following sowing, 100 mL of distilled water was added into every pot. Ten-day-old seedlings were thinned to one plant (the most robust) per pot. The placement of pots in the growth chamber was randomized. Manual watering of each pot with 50 mL of distilled water was conducted every other day. Soil moisture was monitored, and water content was maintained throughout the experiment. The pots were rotated variably once per week. The plants were harvested 8 weeks after sowing.

2.3. Lettuce analysis

Lettuce shoots were severed at ground level, and the roots were gently cleaned of soil and washed with water. The lettuce shoots and roots were dried at 60 °C until a constant weight, and dry aboveground and root biomass were obtained. The chemical digestion of individual samples was followed by analyses of the contents of N, P, K to (Bowman, 1989). The content of N was determined according to (Lu, 1999); K according to (Nowosielski, 1968). All analyses and all measurements were performed using atomic absorption spectrometry (AAS; Agilent 55B AA; Agilent Technologies, CA, USA). The content of P was determined using spectrophotometry (Spectrophotometer: Onda VIS V-10 Plus, Giorgio Bormac, ITA) according to (Olsen and Sommers, 1982).

2.4. Analysis of soils after the experiment

2.4.1. Soil sampling

A mixed soil sample was collected from each pot following lettuce harvesting. These soil samples were homogenized by sieving through 2 mm mesh sieve (Retsch sieve 200 × 50, Retsch, Germany) and divided into several portions for various processing based on determined soil properties. Air-dried sample portions were used to pH analysis (ISO_10390 2005).

2.4.2. Enzyme analysis

Freeze-dried samples were used to analyze enzymatic activities: β-glucosidase (GLU), arylsulfatase (ARS), phosphatase (Phos), urease (Ure), and N-acetyl-β-D-glucosaminidase (NAG) (ISO_20130 2018). The p-nitrophenol (PNP) derivatives of specific soil substrates were employed for Vis spectrophotometric measurement (Infinite M Nano, Tecan Trading AG, Switzerland) at λ = 405 nm (GLU, ARS, Phos, NAG). Urease activity was determined by measuring the amount of ammonium produced from the substrate urea, detected via VIS spectrophotometry using the reagent cyanurate (λ = 650 nm). Each soil sample was measured in nine replicates. Samples stored at 4 °C were utilized for determining dehydrogenase activity (DHA) (Doi and Ranamukhaarachchi, 2009), soil basal respiration (BR), and multi substrate-induced respirations (SIR). DHA was measured using the 2,3,5-triphenyltetrazolium chloride (TTC)-based method. Various respiration types, BR and substrate induced types (SIR) were measured, utilizing D-glucose (Glc-IR), D-trehalose (Tre-IR), N-acetyl-β-D-glucosamine (NAG-IR), L-alanine (Ala-IR), L-lysine (Lys-IR), L-arginine (Arg-IR) and using the MicroResp® device (The James Hutton Institute, Scotland). The final measurement was done on a microplate spectrophotometer (Infinite M Nano, Tecan Trading AG, Switzerland) (Campbell et al., 2003).

2.4.3. DNA analysis of soil microorganisms

DNA extraction was carried out using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA) with 0.5 g of freeze-dried soil sample. The isolated DNA was quantified using Nanodrop One (Thermo Scientific, USA). Real-time qPCR was performed on a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, USA) utilizing the SYBR-Green platform. Partial bacterial (16S rDNA) and fungal (18S rDNA) genes coding for ribosomal RNA in soil DNA extracts were quantified via real-time PCR using the following primers: 1108F (5′ ATGGYTGTCGTCAGCTCGTG 3′) and 1132R (5′ GGGTTGCGCTCGTTGC 3′) for bacteria (Amann et al., 1995), FF390 (5′ AICCATTCAATCGGTAIT 3′) and FR1 (5′ CGA-TAACGAACGAGACCT 3′) for fungi (Vainio and Hantula, 2000).

2.4.4. Thermogravimetry of soil

Thermogravimetry was performed using a thermobalance (TA Instruments Q 550, Delaware, USA). Approximately 200 mg of soil, which had been equilibrated at 43 % relative humidity for 2 weeks to equilibrate the moisture in all soil at the same conditions (Kučerík et al., 2020), was placed on ceramic pans made of Al_2O_3 and heated from 20 to 600 °C in a dynamic air atmosphere, with the heating rate of 10 °C min^{-1}.

The mass loss obtained at specific temperature intervals was evaluated in accordance with previous studies: mass loss from 20 to 200 °C corresponds to moisture content, from 200 to 300 °C to the labile soil organic matter pool, from 300 to 450 °C to the stabilized pool, and from 450 to 600 °C to the stable (refractory) pool (Tokarski et al., 2020).

The residual P3HB was calculated by the difference in mass loss between the amended and respective unamended variants in the temperature interval from 200 to 300 °C, employing the method reported by Palucha et al. (2024).

2.5. Statistical analyses

Data processing and statistical analyses were conducted using R, version 4.3.2 (R_Core_Team, 2023). Principal component analysis (PCA) was done to explain the variation in the selected soil properties. One-way analysis of variance (ANOVA) using type I (sequential) sum of squares at a significance level of 5 % was employed (Zar, 1984) to reveal significant differences between factor level means. Tukey's HSD test was used to calculate factor level means for each treatment at significance level of 5 %. Advanced statistical barplots were created to present the results of the one-way ANOVA and Tukey HSD test (showing statistically significant difference between treatments. A two-way ANOVA with interaction (Type I SSQ) was used to assess the impact of PHB across the treatments. However, for reporting detailed results, only one-way ANOVA was used separately, depending on the first factor (Variants), where the type of sum of squares did not matter. Additional statistical tools were also used, such as the Rohlf biplot for standardized PCA.

Pearson correlation analysis was conducted to reveal the linear dependence between all soil properties (DHA, BR, Glc_SIR, etc.). Specifically, we tested for example the relationship between DHA and BR, between DHA and Glc_SIR, between BR and Glc_SIR, etc. The correlation graphs, which are included in the supplementary materials, clearly illustrate these relationships. The Pearson correlation coefficient was interpreted as follows: $0.0 < r < 0.3$ (negligible correlation), $0.3 < r < 0.5$ (low correlation), $0.5 < r < 0.7$ (moderate correlation), $0.7 < r < 0.9$ (high correlation), and $0.9 < r < 1.0$ (very high correlation) (Hinkle et al., 2003).

3. Results

3.1. Plant biomass and nutrients analysis

In all cases, the addition of P3HB decreased above-ground fresh plant biomass by 86–98 % and dry plant biomass by 81–99.5 % compared to the respective controls. The highest AGB_dry was recorded in the 100_0 control, followed by 80_20 control, which showed 20 % lower values (Fig. 1a, Table S1). Dry root biomass followed the same pattern as AGB_dry (Fig. 1b–Table S1).

Root-to-shoot ratios (R:S) calculated from both fresh and dry plant biomass, exhibited a decreasing trend with an increasing soil-to-sand ratio in unamended (P3HB-free) soil. However, in P3HB-amended soil, this trend was observed only for R:S_dry (Table 1). Among the P3HB amended variants, the 20_80 mixture had the highest R:S_fresh (2.0), whereas variants with a higher soil proportion showed significantly lower ratios. Notably, only the 20_80 P3HB-amended variant had both R:S_fresh and R:S_dry higher than its unamended counterpart. In contrast, for other soil-to-sand ratio, unamended soil variants consistently exhibited higher R:S values than their P3HB-amedned counterparts (Table 1).

The N content in plant biomass was significantly higher ($p < 0.05$) in the 60:40 soil-to-sand ratio with P3HB, followed by 0:100 and 20:80 ratios with P3HB, showing increases of 67 %–43 % (Fig. 1c–Table S1). N_biomass values in P3HB treatments were 15 %–138 % higher compared to their P3HB-less counterparts with the same soil-to-sand ratios. However, P_biomass in P3HB variants decreased by 2–29 % compared to controls (except of 40_60 variant with P3HB). P content in plant biomass was the highest under P3HB amendment in 80_20 (Fig. 1d, $p < 0.05$), followed by 100_0 (Fig. 1d–Table S1). The highest values for K content in plant biomass were found for 40_60 with P3HB and 60_40 with P3HB, which were both 22 % higher than 100_0 control (Fig. 1e–Table S1). K_biomass increased in P3HB variants over P3HB-free variants with equal soil-to-sand ratio by 9–29 %. The 0_100 without P3HB had the highest DM value, followed by unamended 20_80 and 60_40 (14-7 % lower than 100_0, Fig. 1f). Across the P3HB amended variants, 0_100 with P3HB showed significantly highest value, followed by 20_80 with P3HB (11-5 % lower than 100_0, Fig. 1f–Table S1).

3.2. Soil enzyme activities

Dehydrogenase activity peaked in the 100:0 ratio with P3HB, 39 % higher than the control (Fig. 2a, Table S1). Glucosidase activity was highest in control, with the 100:0 ratio with P3HB being 7 % lower (Fig. 2b–Table S1). P3HB had no significant impact on glucosidase activity in other variants.

In contrast, phosphatase activity increased from 7 % to 317 % under P3HB compared to unamended P3HB-less variants (Fig. 2c–Table S1). Urease activity increased by 55–107 %, while chitinase (N-acetyl-β-D-glucosaminidase) increased by 9–40 % (Fig. 2d and e) under P3HB application compared to 100_0 control. The highest urease activity was found in 20_80 with P3HB, followed by 40_60 with P3HB amendment (Fig. 2d–Table S1). Chitinase activity peaked in 100_0 with P3HB, followed by 80_20 with P3HB (Fig. 2e–Table S1).

In a P3HB amended variant, arylsulfatase decreased under higher soil-to-sand ratios, namely in 100_0 was 22 % higher and in 80_20 was 6 % higher compared to the respective P3HB-less variants. However, the opposite trend was observed for lower soil-to-sand ratios, such as ≤ 60 % soil, with increases from 25 to 37 % (Fig. 2f–Table S1). Statistical analysis showed that all enzymes increased with 16S and 18S rDNA in both control and P3HB-amended soil (Table S3).

The C acquisition results revealed an increase in high soil-to-sand ratios. A comparison between P3HB-amended and unamended variants showed that C acquisition was generally lower in the presence of P3HB (Table 2). N acquisition had a decreasing trend with increasing soil-to-sand ratios under unamended conditions (values from 0.85 to

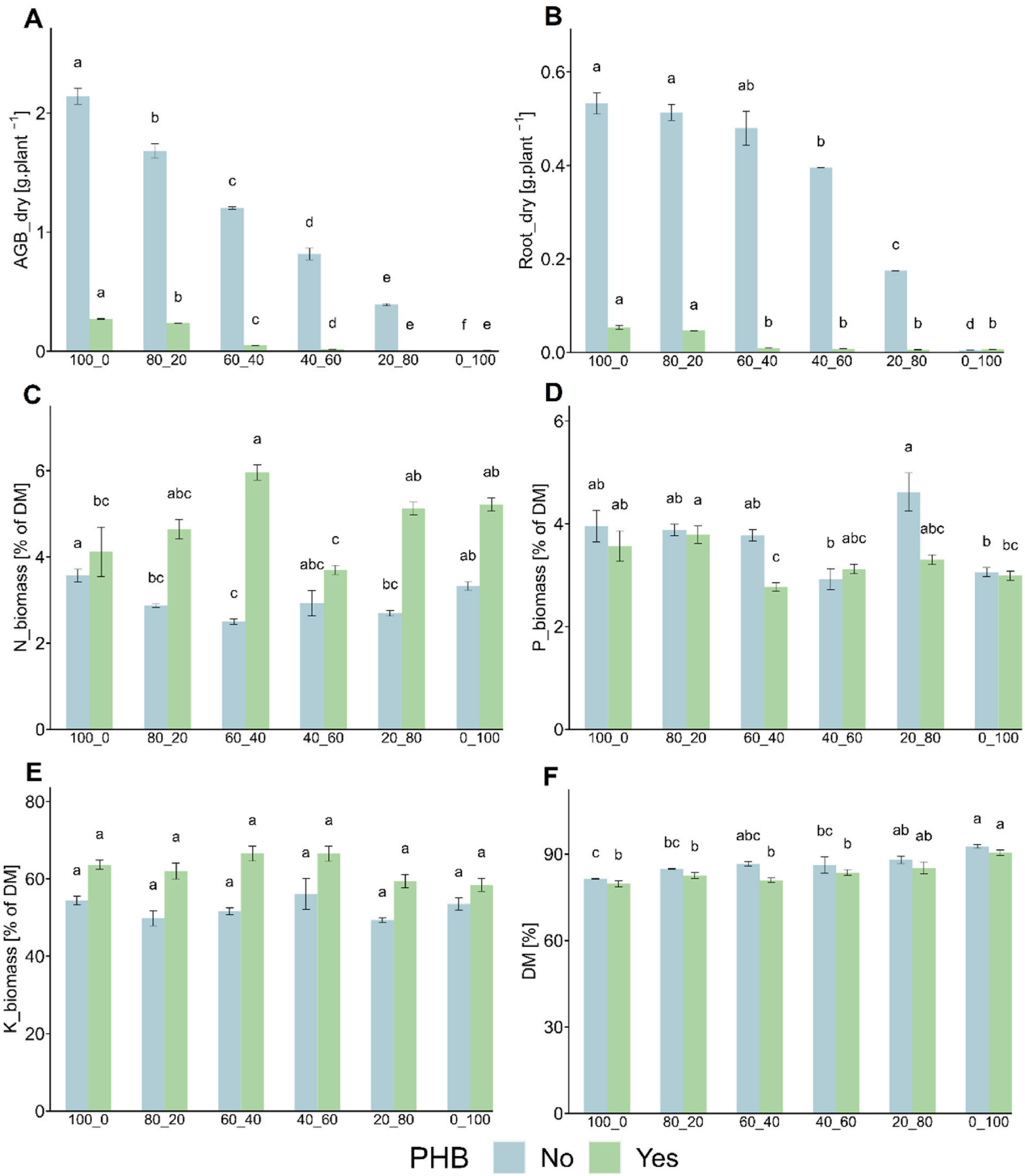

Fig. 1. Dry aboveground (a) and root biomass (b), nitrogen (c), phosphorus (d), potassium (e) content in the dry plant biomass, and dry matter of soil (f) in the variants with varied soil-to-sand ratio, without P3HB ("No") and with P3HB ("Yes") amendment Mean values ($n = 3$) ± standard error of mean (SEM); letters indicate statistical differences between the variants (discriminated as either unamended or P3HB-amended, analyzed by Tukey's HSD test) on the level of significance $p \leq 0.05$.

0.64); under P3HB it was higher compared to unamended variants (0.82–0.73, 22 % higher in average), except for the 0_100 variant (Table 2). The vector values were observed to be higher in the unamended P3HB variants and were directly correlated with the soil-to-sand ratio (Table 2). In the unamended variants, angle values ranged from 53.7° to 55.9°, with the exception of the 80_20 ratio. Conversely, in the P3HB-amended variants, the angle values were consistently lower, ranging from 44.1° to 51.7°.

3.3. Soil basal and substrate induced respiration

Soil basal respiration increased with the addition of P3HB, except in the 60:40 and 40:60 ratios, where it decreased by 23 % and 8 %, respectively, compared to the unamended variants (Fig. 3a). The highest basal respiration was recorded in the 100:0 and 0:100 ratios with P3HB, being 209 % and 202 % higher, respectively, than the variants without P3HB (Fig. 3a–Table S1). This was followed by the 20:80 ratio with P3HB, which was 163 % higher than the control.

Glucose-induced respiration was highest in the 100:0 ratio with

Table 1
Root-to-shoot (R:S) fresh and dry biomass ratios of plants grown in different soil-to-sand mixtures, with and without P3HB amendment.

Variant	P3HB	R:S ratio_fresh	R:S ratio_dry
sand 100 %	No	3.00	–
sand 100 %	Yes	0.67	1.00
soil 20 % + sand 80 %	No	0.49	0.44
soil 20 % + sand 80 %	Yes	2.00	1.00
soil 40 % + sand 60 %	No	0.36	0.49
soil 40 % + sand 60 %	Yes	0.22	0.50
soil 60 % + sand 40 %	No	0.27	0.40
soil 60 % + sand 40 %	Yes	0.22	0.20
soil 80 % + sand 20 %	No	0.18	0.30
soil 80 % + sand 20 %	Yes	0.14	0.21
soil 100 %	No	0.14	0.25
soil 100 %	Yes	0.18	0.19

P3HB, showing a 20 % increase compared to the unamended variant. The next highest values were in the 80:20 ratio with P3HB, which was 12 % higher than the unamended variant (Fig. 3b–Table S1). Similarly, P3HB addition resulted in the highest respiration induced by glucosamine, trehalose, and lysine in the 100:0 and 80:20 ratios (Fig. 3c–e, Table S1). However, arginine-induced respiration was an exception, being highest in the 20:80 ratio with P3HB, with a 200 % increase compared to the unamended variant, followed by the 40:60 ratio with P3HB, which increased by 180 %. In contrast, under low soil content (≤40 %), unamended variants had 46–60 % higher Arg-IR values than P3HB-treated ones.

3.4. Microbial community composition

The dilution of soil with sand reduced the positive effect of P3HB on bacterial biomass (16S rDNA), with similar values observed in the 0:100 and 20:80 ratios (Fig. 4a, Table S1). In contrast, higher 16S rDNA values were seen in the 100:0, 60:40, and 40:60 ratios with P3HB, showing increases of 15 %–46 % compared to the unamended variants, suggesting a positive impact of P3HB on bacterial proliferation. Additionally, 18S rDNA values indicated that P3HB addition stimulated the fungal community, increasing by 11 %–52 % in all ratios except 100:0 and 0:100 (Fig. 4b). The highest 18S rDNA value was recorded in the 80_20 with P3HB, which was 7 % higher than 100_0 with P3HB. Conversely, 100_0 without P3HB had the second highest value, which was higher compared to 100_0 with P3HB (Fig. 4b–Table S1).

3.5. Influence of P3HB on soil fractions stability analysis

Fig. 5 summarizes the data obtained from thermogravimetry, reflecting the content of soil stability fractions. Fig. 5a compares the equilibrium moisture content in soils, as indicated by mass loss 20 and 200 °C. The results indicate a slight increase in soil moisture in 80_20 and 40_60 with P3HB. Fig. 5b presents the quantity of the labile fraction obtained in the interval from 200 to 300° between C. Since P3HB also degrades in this interval, the data suggest that all spiked soils still contain residual P3HB, the quantity of which is discussed in subsequent paragraphs. Fig. 5c summarizes changes in the range of 300–450 °C, representing the so-called stabilized organic matter, which includes an increase in microbial biomass, while P3HB is already degraded in this range. Fig. 5d covers mass loss from 450 to 600 °C, associated with the most thermally and microbiologically stable fraction, or the refractory pool of soil organic matter, showing a slight statistically significant increase after adding P3HB in the 80:20 ratio. The terms “stabilized organic matter” and “refractory organic matter” refer to different thermostable fractions of SOM, as defined in the Materials and Methods section 2.4.4. These classifications are based on thermogravimetric analysis of soil organic matter, following the methodology established in previous study by Tokarski et al. (2020) and further used e.g. by Palucha et al. (2024).

3.6. Residual content of P3HB

The residual P3HB was determined as the difference in mass losses between the variant with and without P3HB amendment in the temperature range from 200 to 300 °C (Fig. 6). It is evident that in 100_0, there was still approximately 42 % of residual P3HB. Increasing the sand content in 80_20 variant resulted in a slight decrease in P3HB residue to 34 %. Subsequently, the values increased to 41 % in 60_40, to 50 % in 40_60, and to 53 % in 20_80.

4. Discussion

4.1. Plant growth and nutrient contents

Previously, it was shown that intact BDP and its decomposition products posed no toxicity to cells and had low cytotoxicity (Napathorn, 2014). However, recent studies have challenged this notion, particularly concerning the mutual interaction between BDP, soil microbiota, and plants growing in BDP-contaminated soil (Qi et al., 2020; Zang et al., 2020). Serious P3HB-related plant dying of wheat (*Triticum aestivum* L.) (Zhou et al., 2021), and the suppression of growth of lettuce (Brtnicky et al., 2022) were reported. Such adverse effects were ascribed to PHBV-(P3HB-) induced SOM deterioration, driven by intensified N and P cycling, likely leading to their rapid exhaustion. This hypothesis couples increased nutrient losses with excessive mineralization and microbial utilization of plastic-derived organic carbon, which is accompanied by an enhanced concurrent uptake and immobilization of P and N in microbial biomass (Meng et al., 2022). As a result, these nutrients became inaccessible to plants leading to the observed reduction in plant growth in the current study.

The increased 16S and 18S rDNA levels in P3HB-amended variants implies that the altered P3HB-associated microbiome assimilated more nitrogen into microbial biomass (Brtnicky et al., 2024b), thereby reducing nitrogen uptake by plants and leading to lower plant biomass production (as evidenced by decreased AGB dry weight). This mechanism, where higher microbial abundance, activity, and growth in the rhizosphere intensify microbe-plant competition for nutrients, has been previously reported (Blagodatskaya et al., 2010). The increased microbial demand for avalable C in response to P3HB likely enhanced nutrient transformation, reducing their availability for plant uptake into the rhizosphere (Kuzyakov, 2006; Nguyen, 2003). In contrast, reduced nitrogen availability in soil lead to a significant accumulation of this limiting nutrient in the aboveground plant biomass.

The present study confirmed the negative impact of P3HB on plant biomass (Fig. 1), which is in accordance with our previous study (Brtnicky et al., 2022), where the suppression was found regardless of the soil used for the test or the soil-to-sand ratio. This indicates that the deleterious effects were not dependent on the SOM content. Eight-week preincubation of P3HB-amended soil before lettuce sowing agreed with the duration of the most intensive BDP degradation in the soil (Šerá et al., 2020). During the initial degradation, the lag phase refers to the period when microorganisms adapt to a new environment and colonize the surface of bioplastics, and visible degradation is minimal. Once the lag phase ends, microbial activity increases, leading to the degradation of the bioplastics at a measurable rate (Tokiwa et al., 2009). In this work, the increase in microbial activity is evident from the comparison between the values of dehydrogenase and basal and induced respirations (Fig. 2) and values of residual P3HB content (Fig. 6). The rapid degradation of P3HB upon introduction into the soil and the depletion of available nutrients by growing microorganisms stimulate early competition between plants and microbial communities for nutrients and suppress plant growth (Brtnicky et al., 2024). BDP addition also decreases soil water retention, bulk density, and total porosity (Jiang et al., 2017), thus affecting water and nutrient absorption by plants (Zhang et al., 2020; Fojt et al., 2022). Soil saturation with water was reduced by the addition of sand (Fig. 5a), and the SOM presumably also decreased,

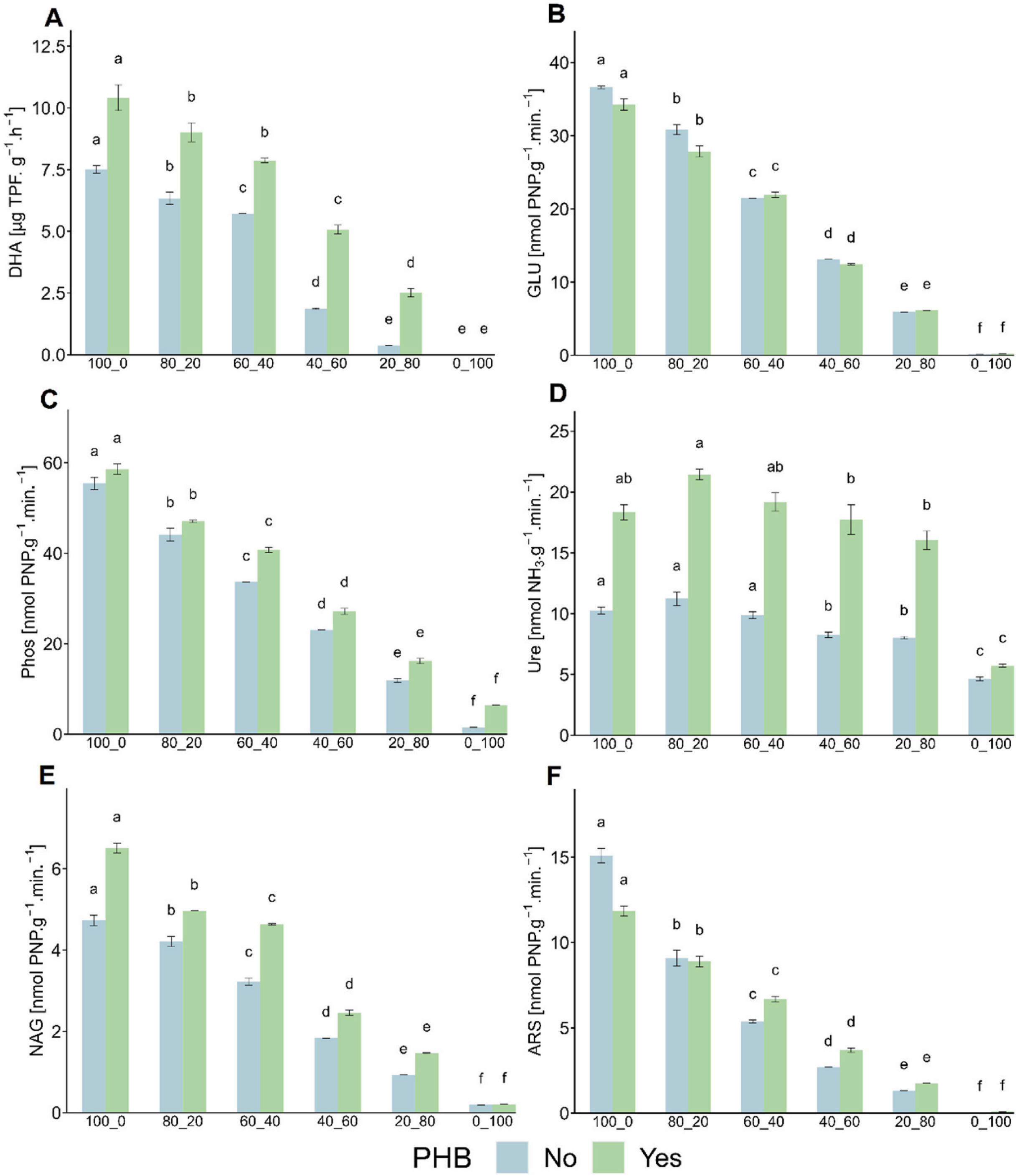

Fig. 2. Soil enzyme activities in the variants with varied soil-to-sand: ratio, without P3HB ("No") and with P3HB ("Yes") amendment Dehydrogenase (DHA, a), B-glucosidase (GLU, b), phosphatase (Phos, c), urease (Ure, d), N-acetyl-β-D-glucosaminidase (NAG, e), arylsulfatase (ARS, f); mean values (n = 3) ± standard error of mean (SEM); letters indicate statistical differences between the variants (discriminated 0as either unamended or P3HB-amended, analyzed by Tukey's HSD test) on the level of significance $p \leq 0.05$.

while DM increased (Fig. 1f). This result suggests that soil variants with high sand contents may be more vulnerable to desiccation, which could be intensified by the presence of P3HB (Wan et al., 2019; Fojt et al., 2022). Nevertheless, a comparison of amended and unamended variants revealed that the moisture content slightly increased in amended variants (Fig. 5a), likely because the P3HB biodegradation is associated primarily with the formation of biofilms on BDP surfaces that increases their hydrophilicity and water retention (Han et al., 2020).

The addition of biodegradable polymers such as P3HB and PHBV to soil could potentially enhance the transformation and subsequent rapid depletion of N and P by soil microorganisms, thereby causing stress for plants (Boots et al., 2019; Zhou et al., 2021; Brtnicky et al., 2022). The decreased availability of nutrients in P3HB-amended soil affected their content in plant biomass, which was reflected in the yield of AGB_dry and Root_dry (Table S3). However, in line with our previous study (Brtnicky et al., 2022), it was confirmed that the addition of sand to soil can mitigate the adverse effects of P3HB on nutrient uptake by plants and the content in dry biomass. The observed high N accumulation in

Table 2
Soil enzyme activities, nutrient acquisition ratios, and limitation indicators in different soil-to-sand mixtures, with and without P3HB amendment.

	DHA	GLU	NAG	Ure	Phos
	(mg TPF·g^{-1}·h^{-1})	*(nmol PNP·g^{-1}·min^{-1})*		*(nmol NH$_3$·g^{-1}·min^{-1})*	*(nmol PNP·g^{-1}·min^{-1})*
sand 100 %/+ P3HB	0.00/0.00	0.16/0.21	0.19/0.20	4.68/5.75	1.52/6.34
soil 20 % + sand 80 %/+ P3HB	0.38/2.51	5.89/6.31	0.94/1.46	8.09/16.23	11.72/16.23
soil 40 % + sand 60 %/+ P3HB	1.87/5.08	12.98/12.39	1.84/2.44	8.30/17.52	23.31/27.39
soil 60 % + sand 40 %/+ P3HB	5.71/7.87	21.38/21.98	3.24/4.63	10.03/19.46	33.86/41.32
soil 80 % + sand 20 %/+ P3HB	6.33/9.01	30.74/28.05	4.12/5.08	11.33/21.65	43.60/47.48
soil 100 %/+ P3HB	7.51/10.41	36.88/34.45	4.65/6.51	10.46/18.73	55.14/59.14
	C acq	*N acq*	*C, N, P limitation vector*	*angle/rad*	*° (angle degrees)*
sand 100 %/+ P3HB	-/-	0.85/0.71	4.53/1.22	-/-	-/-
soil 20 % + sand 80 %/+ P3HB	0.67/0.66	0.73/0.82	1.12/1.09	0.84/0.77	48.20/44.13
soil 40 % + sand 60 %/+ P3HB	0.84/0.79	0.66/0.78	1.45/1.29	0.94/0.84	53.66/47.87
soil 60 % + sand 40 %/+ P3HB	0.89/0.85	0.67/0.76	1.58/1.40	0.94/0.86	53.72/49.47
soil 80 % + sand 20 %/+ P3HB	0.91/0.87	0.67/0.76	1.63/1.44	0.94/0.87	54.05/49.60
soil 100 %/+ P3HB	0.93/0.89	0.64/0.73	1.69/1.50	0.98/0.90	55.90/51.65

Carbon acquisition ratio (Cacq.), nitrogen acquisition ratio (Nacq), Vector (C limitation index), Angle (N, P limitation index).

plant biomass in sand-amended soil under P3HB treatment (in 60_40, 20_80, and 0_100) supports the hypothesis of adaptive accumulation of limiting nutrients due to their deficiency. The increased K_biomass in P3HB-amended variants under various soil-sand combinations in the present study (Fig. 1e–Table S1) aligns with the known role of potassium (K) in plant responses to stresses (Mostofa et al., 2022). Therefore, this finding supports the assumption of increased plant stress due to the deteriorating impacts of P3HB in soil. It is noteworthy that P3HB is only one of many biodegradable plastic mulching films on the market (Qi et al., 2018). Consequently, it is necessary to evaluate the performance and potential impact of these plastics on soil and plant health before widespread adoption. Despite high expectations for these plastics, the study results suggest that caution should be taken when using them without proper investigation.

4.2. Soil microbial biomass and activity

Low plant growth but high microbial activity following the addition of PHBV and P3HB to soil have been reported (Zhou et al., 2021; Brtnicky et al., 2022). The activities of dehydrogenase, chitinase (at high soil: sand ratio), phosphatase (at low soil: sand ratio), and especially urease increased in P3HB-amended variants compared to unamended ones (Brtnicky et al., 2022). The enhancement of enzyme activities was more pronounced in soils with P3HB than without, as observed for all enzymes except glucosidase (Fig. 2, Table S1). This indicates a high proliferation of microbial populations and activities of dehydrogenase and arylsulfatase associated with the increasing sand content. These results align with the assumption that, under low access to readily available nutrients and SOM, P3HB-induced consumption of C sources (such as BDP) promotes the mining of organic sources of the respective nutrients. High dehydrogenase activity indicates an intensification of SOM degradation due to using P3HB as a C source (Bonartseva et al., 2003; Rizzarelli et al., 2004; Zhou et al., 2021). A notable increase in P3HB-mediated dehydrogenase activity in the 90_10 with P3HB reported in (Brtnicky et al., 2022) indicated the utilization of the sole C source (P3HB). However, the eight-week preincubation of the substrate before the lettuce sowing enabled microbial growth on C-substrate (P3HB), leading to the partial transformation of BDP.

Consequently, a decrease in the initial boost of dehydrogenase was observed as the formed C-compounds were further utilized under the catalysis of other soil enzymes. This assumption of a mitigated boost in C utilization due to the eight-week preincubation was supported by the values of the C acquisition ratio (Table 2), which were directly dependent on the soil: sand ratio (i.e., on SOM content). The unamended variants showed higher values (C acquisition ratio and C limitation-indicating *vector* value) than P3HB treatments, suggesting a higher demand for microorganisms for C in the soil without BDP. Lower values of glucosidase activity under P3HB (at high soil-to-sand ratios 80:20 and 100:0) compared to variants without P3HB might be attributed to the preferential utilization of P3HB over simple sugars from indigenous SOM (Ferraz-Almeida et al., 2015).

High activities of N-acquisition enzymes reflected that N could become a limiting nutrient for microbial proliferation in the presence of P3HB (Mooshammer et al., 2014a, 2014b) (Table 2). The N acquisition values in all P3HB-amended variants were higher than in unamended ones (under the same soil:sand ratios). In comparison, low angle values for unamended variants (in soil with P3HB, closer to the value of 45°) indicated a tendency towards N rather than P limitation (Table 2). Moreover, the rise of urease activity in the 80_20 with P3HB compared to the 80_20 indicated a high urease content in soils. Enhanced activities of phosphatase and arylsulfatase P3HB-amended soil (Fig. 2, Table S1) were coupled with the rise of bacterial (Phos: 0.91, ARS: 0.87) and fungal (Phos: 0.95, ARS: 0.94) abundances (Fig. 4a and b).

High SIRs were observed under P3HB addition, aligning with previous findings (Zhou et al., 2021) and possibly be associated with the stimulation of microbial growth (Fig. 4a and b, Table S1) and enzymatic activities (Fig. 3). These findings were further supported by a strong positive relationship between microbial biomass, enzyme activities and soil respiration (Table S3, Fig. S4). Nevertheless, all induced respiration types (except Arg-IR) depended on the soil:sand ratio in unamended and P3HB-amended variants, contrasting with the previous study results (Brtnicky et al., 2022). Induced SIR in P3HB-amended soil increased with the sand content, indicating strong degradation of P3HB under high soil aeration. However, the plant-deteriorating effect was mitigated by the eight-week preincubation period, as shown by the activities of C enzymes. These results confirm the hypothesis that P3HB addition suppresses lettuce growth by changing plant nutrition. The changes are caused by nutrient limitation due to BDP-induced enhancement of soil microbial activity and biomass increase, both of which are soil composition- and time-dependent.

4.3. Residual P3HB and SOM dynamics

The content of individual fractions of SOM confirmed the findings on increased microbial activity in soils amended with P3HB (Fig. 5). This increase was particularly noticeable in the 300–450 °C temperature range, corresponding to the stabilized SOM fraction (Tokarski et al., 2020). Notable increases in this fraction were observed in the 60_40, 80_20 and 100_0 variants and correlated with microbial biomass (Brtnicky et al., 2022). The relative increase in the stabilized fraction was the highest in the 80_20 variant, which had similar aboveground biomass dry weight and root dry weight parameters as in the 100_0, both

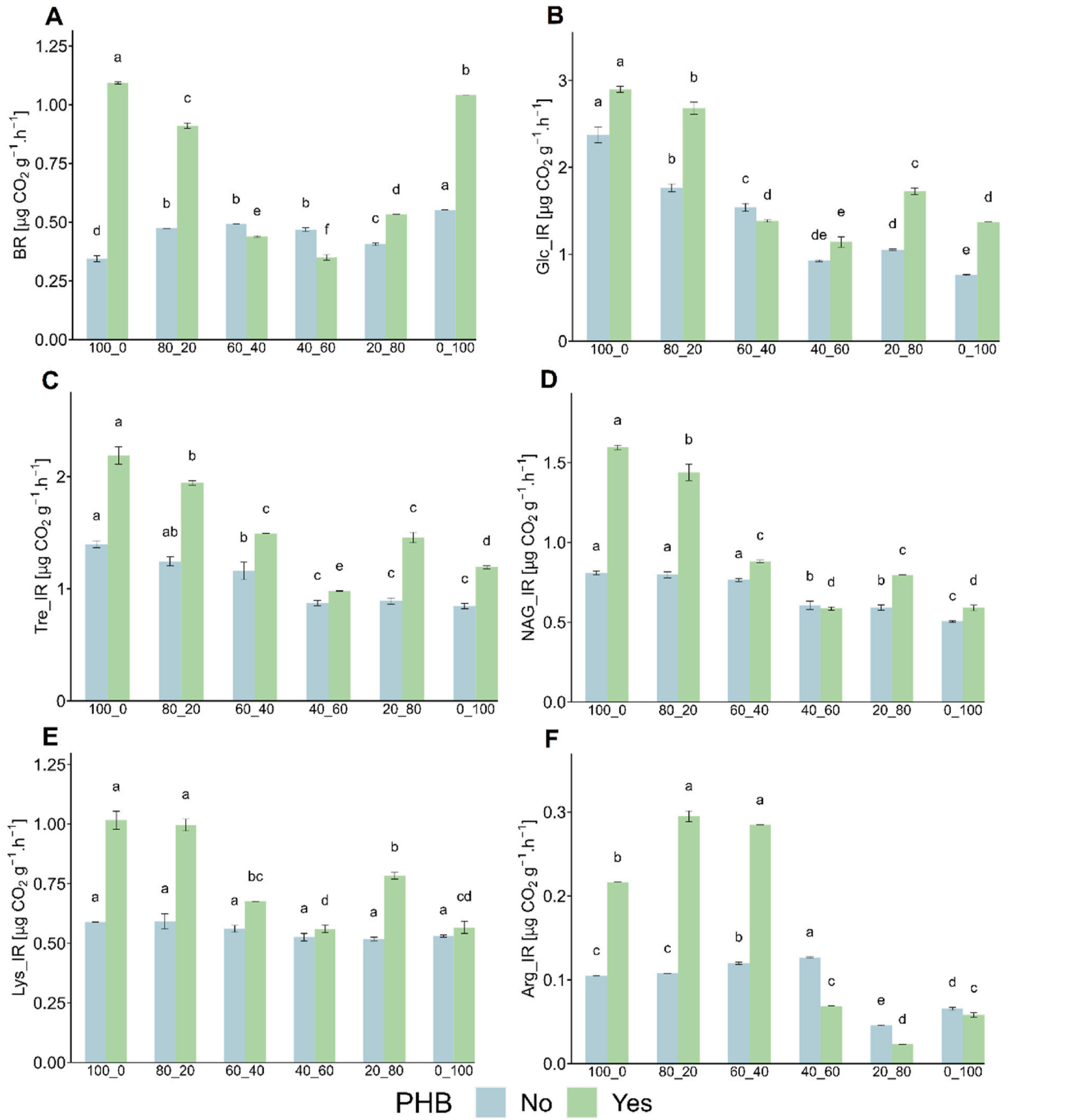

Fig. 3. Soil basal (BR, a) and substrate induced respiration types (IRs) in the variants with varied soil-to-sand ratio, without P3HB ("No") and with P3HB ("Yes") amendment. IR by D-glucose (Glc-IR, b), D-trehalose (Tre-IR, c), N-acetyl-β-D-glucosamine (NAG-IR, d), L-lysine (Lys-IR, e), L-arginine (Arg-IR, f); mean values ($n = 3$) ± standard error of mean (SEM); letters indicate statistical differences between the variants (discriminated as either unamended or P3HB-amended, analyzed by Tukey's HSD test) on the level of significance $p \leq 0.05$.

variants under P3HB amendment.

The rise in the stabilized SOM fraction, which aligns with the observation that the greatest degradation of P3HB occurred in the 80_20, suggests potential implications of the research. This outcome supports the assumption that P3HB-derived carbon enhances overall SOM turnover, leading not only to the mineralization of plastic-derived carbon, but also to an accelerated breakdown of intrinsic labile C fractions. Consequently, this process results in the proportionally higher content of stabilized organic carbon in P3HB-amended soil. This finding, even more pronounced than in the 100_0, indicates that 20 % dilution with sand enhances aeration and nutrient transport, thus, promoting biodegradation processes (Altaee et al., 2016) and highlights oxygen as a critical factor in explaining the fate of P3HB in soil. The potential implications of this research extend to plant biology, as it is hypothesized that suppressed growth in lettuce could be due to inhibited root respiration, impacting the plant's growth and morphology (Pineda et al., 2020). The comparable Lys-IR and higher Arg-IR values in 80_20 vs 100_0 suggest that the addition of sand can promote the degradation of bioplastics, enabling enhanced microbial activity, which competes with root respiration, thus confirming the hypothesis of lettuce growth suppression. This enhanced microbial activity, driven by the activation and multiplication of bacteriome and fungiome aligns with previous findings

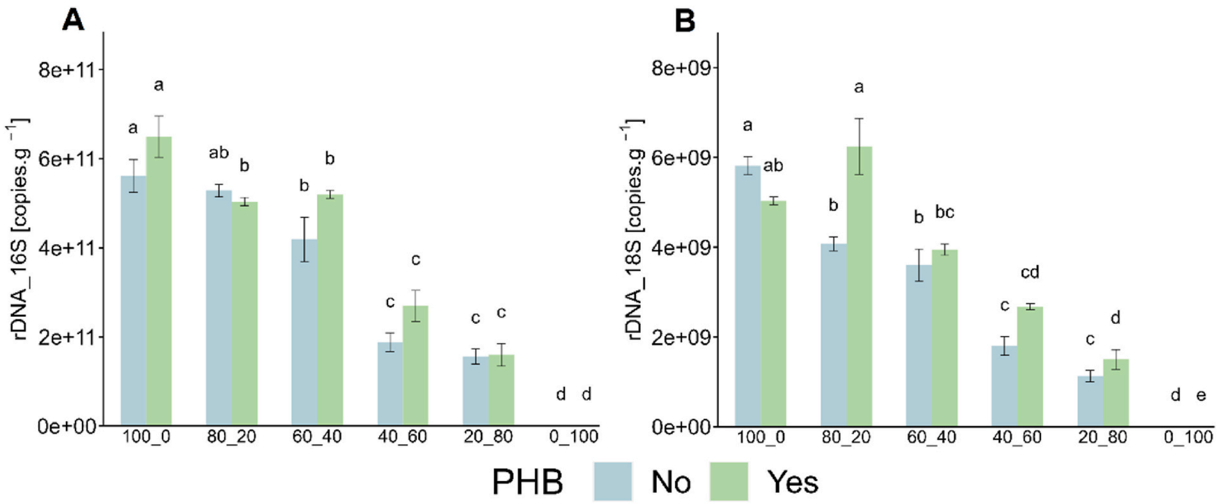

Fig. 4. Soil microbial biomass of bacteria (16S rDNA, a) and fungi (18S rDNA, b) in the variants with varied soil-to-sand ratio, without P3HB ("No") and with P3HB ("Yes") amendment. Mean values (n = 3) ± standard error of mean (SEM); letters indicate statistical differences between the variants (discriminated as either unamended or P3HB-amended, analyzed by Tukey's HSD test) on the level of significance $p \leq 0.05$.

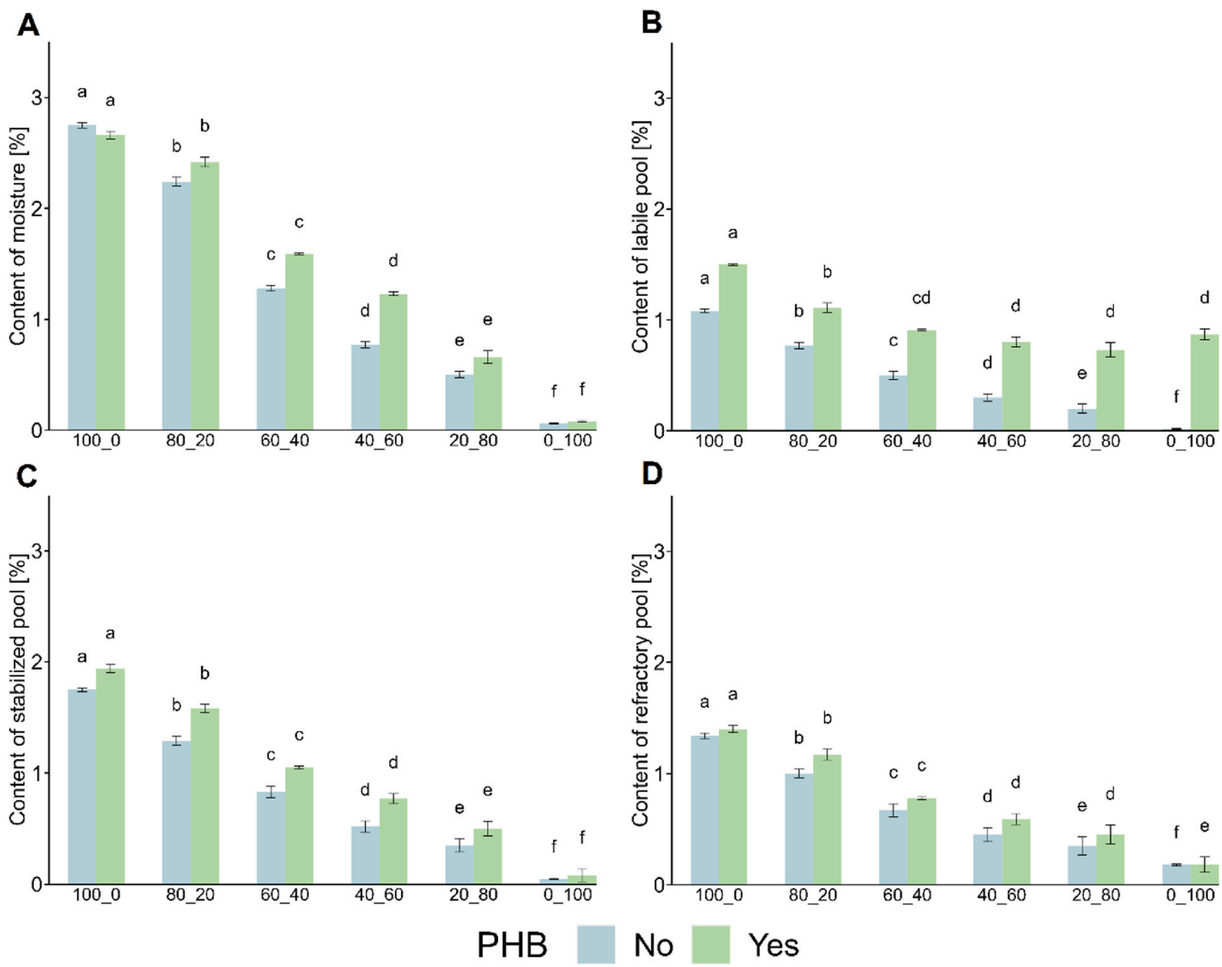

Fig. 5. Soil fractions after P3HB amendment and cultivation of lettuce in the variants with varied soil-to-sand ratio, without P3HB ("No") and with P3HB ("Yes") amendment. (a) Moisture = mass loss from 20 to 200 °C; (b) labile pool = mass loss from 200 to 300 °C; (c) stabilized pool = mass loss from 300 to 450 °C and (d) refractory pool = mass loss from 450 to 600 °C. Mean values (n = 6) ± standard error of mean (SEM); letters indicate statistical differences between the variants (discriminated as either unamended or P3HB-amended, analyzed by Tukey's HSD test) on the level of significance $p \leq 0.05$.

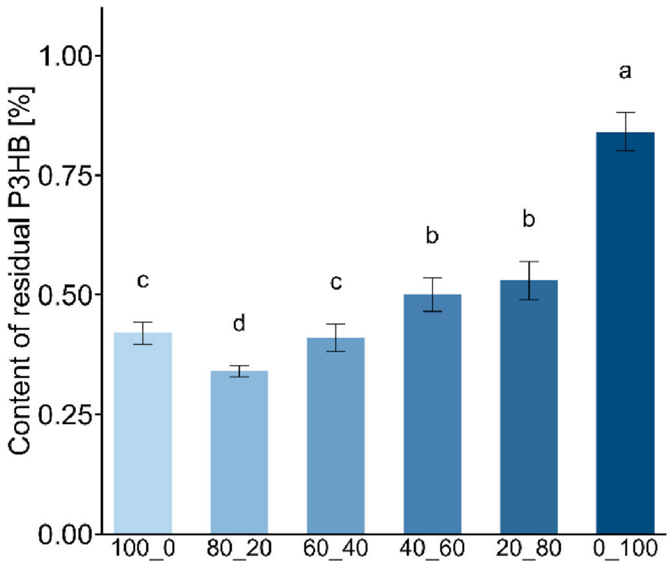

Fig. 6. Residual P3HB after 8 weeks of resting and 8 weeks of cultivation of lettuce in the variants with varied sand:soil ratio. Mean values (n = 6) ± standard error of mean (SEM); letters indicate statistical differences between the variants (discriminated as either unamended or P3HB-amended, analyzed by Tukey's HSD test) on the level of significance $p \leq 0.05$.

onP3HB-induced proliferation of saprophytic fungi in soil (Janczak et al., 2020). This observation is further supported by reportes on the ability of several abundant microbial taxa to catabolize P3HB (Altaee et al., 2016; Sang et al., 2002). Due to their different metabolic and physiological characteristics, fungi are more sensitive to soil oxygen depletion than bacteria. Fungi typically require more oxygen for their metabolic processes than many types of bacteria (Jacoby et al., 2017). The increase in the relative abundance of fungi (Fig. 4a and b) in the 80_20 raises a hypothesis about the significant contribution of soil oxygen content to P3HB biodegradation.

The discussion above aligns with the characteristics of soil types categorized by their texture. The original soil, identified as silty clay loam, is highly fertile due to its substantial nutrient content and high water-holding capacity. However, soil density can impede drainage, leading to reduced aeration (Wei et al., 1985). Modifying the original soil by adding 20 and 40 % sand changed the texture to loam type (variants II and III), creating optimal moisture conditions for plant growth (Geering and Bing So, 2017). Further dilution, creating sandy loam and loamy sand (variants IV and V), enhanced aeration but reduced nutrient levels. Consequently, these modifications highlight the optimal conditions for P3HB biodegradation in soil, while still maintaining, at least to some extent, suitable conditions for plant growth (Schjønning et al., 1994). Moreover, they offer practical insights for optimizing biodegradation tests in soils (e.g. ASTM 5988 or ISO 17556), which currently provide only vague guidelines for selecting soil types. Finally, the soil dilution serves as a practical model for assessing processes in soils that have deteriorated due to elevated temperatures, erosion or poor soil management.

5. Conclusion

Using P3HB in agriculture soil enhanced soil microbial activity, including the growth of microorganisms and the production of exoenzymes, likely due to increased mineralization of native SOM through co-metabolism with P3HB. This finding is important because microorganisms in agricultural soil are typically C-limited, and even small C inputs can impact the microbial community. Furthermore, enzymes such as dehydrogenase, urease, and chitinase were stimulated after adding P3HB, indicating the development of specific microbial groups that degrade bioplastics. However, the positive effects on soil microorganisms were outweighed by adverse effects on plant growth, suggesting that while microbial communities can break down bioplastics, this process may compromise plant nutrient availability and hinder growth. These findings highlight the importance of considering soil texture when modeling plastic degradation, as the byproducts can affect soil processes and plant growth.

CRediT authorship contribution statement

Martin Brtnicky: Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Adnan Mustafa:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Jiri Holatko:** Software, Formal analysis, Data curation. **Anna Gunina:** Writing – review & editing, Validation, Methodology. **Gabrijel Ondrasek:** Writing – review & editing, Software. **Muhammad Naveed:** Writing – review & editing, Formal analysis. **Tereza Hammerschmiedt:** Writing – review & editing, Methodology, Data curation. **Eliska Kamenikova:** Formal analysis, Data curation. **Saud Alamri:** Writing – review & editing, Project administration, Funding acquisition. **Manzer H. Siddique:** Writing – review & editing, Project administration, Funding acquisition. **Antonin Kintl:** Software, Formal analysis, Data curation. **Tivadar Baltazar:** Software, Formal analysis. **Ondrej Malicek:** Validation, Data curation. **Jiri Kucerik:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121618.

Data availability

Data will be made available on request.

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PŘÍLOHA F

Biodegradable microplastics impact on soil: How poly-3-hydroxybutyrate alters microbial diversity and nitrogen mineralization processes

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Abstract

Background: Poly-3-hydroxybutyrate (P3HB) belongs to the group of biodegradable plastics (BPs), which may have adverse effects on plant growth and soil. This study aimed at P3HB microplastics-induced changes in soil microbiome and its activity related to nutrient content and transformation processes, which may explain the deterioration of plant growth. Pot experiment of soil contaminated with P3HB in five different doses, with and without maize, was carried out. Soil mineral N forms and microbial properties as well as plant biomass were determined.

Results: Increasing P3HB content significantly changed the soil microbiome. The fungal community was more sensitive to P3HB compared to bacterial one. However, both communities showed critical P3HB-derived shifts in several taxonomical groups: the increase in (i) P3HB degraders, e.g. bacteria *Actinobacteria* + fungi *Tetracladium*, and (ii) anaerobes (*Clostridium*), and the decrease in (i) nitrifiers *Nitrososphaeria* and *Nitrospiria* and (ii) oligotrophic *Vicinamibacteria* and *Thermoleophilia*. The associated alteration in the metabolism of soil nutrients arising from P3HB-derived C caused over-enhanced nutrient consumption coupled with (i) boosted respiration resulting in partial anaerobiosis, (ii) inhibited N mineralization and (iii) depletion of NO_3-N and the inhibition of maize growth.

Conclusions: The results indicate that soil content of P3HB microplastics exceeding 1% may cause serious damage to soil health and fertility.

Keywords: Biodegradable plastics; Bacteria; Fungi; Nitrification; Soil nitrogen

List of abbreviations:

AGB – aboveground biomass (dry weight), AOB – ammonia oxidizing bacteria, Arg-IR – soil respiration induced by L-arginine, BPs - biodegradable plastics, CCA - Canonical correspondence analysis, Cit-IR – soil respiration induced by citric acid, c(P3HB) – soil content of poly-

3-hydroxybutyrate, DM – dry matter, NAG – soil N-acetyl-β-D-glucosaminidase, NAG-IR – soil respiration induced by N-acetyl-β-D-glucosamine, NH_4-N – soil ammonium nitrogen, N_{min} – soil inorganic nitrogen, NO_3-N – soil nitrate nitrogen, P3HB – poly-3-hydroxybutyrate, PCoA - Principal coordinate analysis, PHAs – polyhydroxyalkanoates, phaZ – qPCR-detected microbial degraders of P3HB, PHBV – poly(3-hydroxybutyrate-co-3-hydroxyvalerate), Tre-IR – soil respiration induced by D-trehalose.

1. Background

Plastic pollution belongs among the most urgent global environmental threats. Replacement of conventional plastics with biodegradable alternatives seems to be one of the perspective solutions Freda. Polyhydroxyalkanoates (PHAs) are a group of natural biodegradable plastics (BPs) which are considered highly advantageous for their physico-chemical and mechanical properties as well as feasibility of their aerobic or anaerobic biodegradation [1,2]. PHAs, which include the most versatile and widely used BPs polyhydroxybutyrates (PHBs), such as poly-3-hydroxybutyrate (P3HB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), are biosynthesized from various carbon (C) substrates by bacteria [3]. Despite their promising biodegradability, increasing use of PHAs in agriculture raises their concentration in soils with residual concentrations potentially exceeding 1.5% [4]. This leads to concerns about their impact on agroecosystems.

So far, adverse effects of PHAs on soil were reported rarely and mostly as less severe (Table 1). Therefore, it is currently difficult to classify them as pollutants. However, after 2021 they appear regularly and gradually uncover possible impacts manifested especially in soil microbiome. The question of whether they should be classified as short-lived pollutants therefore arises.

Soil microorganisms may be affected by various PHA-induced changes in soil environment (Table 1). The effects on soil were coupled with significant and essential shifts in the soil taxonomic and functional diversity as well as microbial community composition [5-8]. Enrichment of soil with PHA-degrading taxa such as *Alphaproteobacteria* [9-10], *Actinobacteria* [9-11], *Ascomycota* [10] and presumably also *Gammaproteobacteria* [12] is coupled with probable abundancy shifts in functional types of microbes such as copiotrophs [13], oligotrophs [14], nitrifying prokaryotes [15], *Firmicutes* [16] and others. Furthermore, several fungal taxa were recognized as PHA degraders with a possible decisive role in their degradation [17]. These are mostly included in the phyla *Ascomycota* [17-19], *Basidiomycota* [18] and *Deuteromycetes* [20].

Table 1 Potentially adverse effects of PHA types in soil

Effect	PHA type			Reference
	PHB	**P3HB**	**PHBV**	
Acidification			•	[8]
		•		[21]
Modification of SOM* structure and stability		•		[22]
Decrease in SOM water binding		•		[22]
Enhanced SOM degradation coupled with C and nutrient turnover			•	[5, 8]
		•		[4, 23, 24]
Decrease of NO_3-N	•			[25]
Decrease of available NO_3^- and NH_4^+ in soil solution			•	[5]
Change in the nutrient balance			•	[26]
		•		[21]
Increase in lead (Pb) availability	•			[25]
Soil microbial community change		•		[23, 24]
			•	[5, 7, 8]

* SOM = Soil organic matter

Although the direct effect of PHAs on soil microorganisms is possible to evaluate as harmless or even positive from a certain perspective, the related changes can have some negative impacts on the entire soil environment. These changes caused imbalances in nutrient transformation rates and their availability [23] and have also been shown to be harmful to plants [5, 23, 27] although no phytotoxic effect on aquatic plants has been proven at least in the case of P3HB [28]. Questions arise – what are the key processes behind the changes? What specific changes occur in the microbiome? And what do they mean for agricultural yield? What are the critical values of PHAs in agricultural soils? Following on from previous research by the author's collective in this topic [4, 21, 22, 23, 28], this study focused on P3HB, and the questions mentioned.

A serious negative impact of P3HB on plants was attributed to the change in the nutrient balance and their availability for plants. P3HB contamination in soil represents an easily utilizable C source affecting accessibility and utilization of intrinsic SOM by soil microorganisms [23]. The input of such a source shifts the C:N ratio out of the optimal stoichiometry (C:N 7–8.6) for soil microorganisms [29, 30]. The resulting increased C:N leads to an increased microbial demand for N followed by N-mining from SOM via enhanced production of N-hydrolase in order to restore the C:N balance [31]. Therefore, rapid utilization of P3HB by the microbial community results in an increased specific microbial metabolism and growth rates coupled with intensified nutrient uptake [8, 23]. This action probably depletes soil nitrogen (N) for plants and strongly inhibits their growth [5, 23, 32]. Although this hypothesis has already been postulated, no one has yet verified it by examining the concentrations of N forms in soil contaminated with P3HB.

Despite many theories, no focused and comprehensible study has yet investigated P3HB-related changes in soil microbiome in detail. In general, the recent level of knowledge suggests that P3HB microplastics trigger a switch of soil fluxes from C, N and other nutrient sequestration into SOM to strongly enhanced consumption with a critical impact on soil N [23]. This is likely coupled with significant changes in microbial activity, diversity and metabolism, leading to negative effects on plant nutrition and growth. Nevertheless, there is still no clear knowledge about the (I) P3HB microplastics-induced changes in microbial community composition related to the changes in microbial activity and (II) nutrient content and changes in transformation indicators, which are related to the deterioration of plant growth. Therefore, this study aimed to contribute to fill this knowledge gap considering different doses of P3HB. The following hypothesis were postulated:

I. The increasing P3HB content in soil modifies microbial community towards lower diversity.
II. The input of labile P3HB is followed by an increased abundance of copiotrophic P3HB degraders and a decreased abundance of nitrifying microorganisms and oligotrophs.
III. P3HB degradation raises microbial N acquisition leading to soil N depletion and reduced N availability to plants.

2. Materials and methods

2.1 Experimental design and sampling

The growth substrates, used for the pot experiment, were prepared by mixing an arable soil sieved through 2 mm mesh sieve with P3HB powder (< 80 μm particle size) in 5 variants based on the share of P3HB: 0%, 0.1%, 1%, 5% and 10% w/w. P3HB content in agricultural soils with P3HB mulching sheets can range from approximately 0.5% to 1.5%, but it is expected to rise in the future [4]. Therefore, P3HB content (c(P3HB)) used can be classified as low (0.1%), medium (1%) and high (5%, 10%); the 0% variant is the control.

The soil was classified as a silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification) sampled (0–15 cm) near the town Troubsko, Czech Republic (49°10'28"N 16°29'32"E). The chemical properties of the soil were: total C 14.0 $g \cdot kg^{-1}$, total N 1.60 $g \cdot kg^{-1}$, P 0.10 $g \cdot kg^{-1}$, S 0.15 $g \cdot kg^{-1}$, Ca 3.26 $g \cdot kg^{-1}$, Mg 0.24 $g \cdot kg^{-1}$, K 0.23 $g \cdot kg^{-1}$; pH

($CaCl_2$) 7.3. P3HB powder (ENMAT Y3000) from TianAn Biologic Materials Co., Ltd. (Ningbo City, China) was used for this experiment. P3HB is slightly hydrophobic polymer having the contact angle between 70° and ≈81°. Further specification of used P3HB can be found in the study of Fojt et al. [22].

Experimental plastic pots (2 L) were filled up with 1.7 kg of the respective substrates. Each treatment was prepared in 10 repetitions (pots). Then, 5 pots of each treatment were sown with sprouted maize seeds (*Zea mays* L.) and five were run without plants. All experimental pots were placed randomly into growth chamber (CLF Plant Climatics GmbH, Germany), where controlled conditions were maintained: 12 hours long photoperiod, light intensity 20 klx, temperature (day/night) 20/12 °C, relative air humidity (day/night) 45/70%, moisture level 65% of water holding capacity. After 90 days, tested plants were harvested at ground level, their height was measured and then the plant biomass was dried at 60 °C to the constant weight to determine the yield of aboveground biomass (AGB) in dry weight from each pot. Further, soil samples were taken from all experimental pots.

2.2 Soil chemical and biochemical analyses

The soil samples were taken as composited from 3 probes per each pot, homogenized by sieving through 2 mm mesh sieve and directly used (fresh samples), stored at 4 °C (in refrigerator) or freeze-dried and stored at -20 °C (in a freezer).

The fresh samples were used for determination of mineral N, NO_3^- (mercurous sulphate electrode type RME 121; Monokrystaly Turnov, Czech Republic) and NH_4^+ (with ammonia gas electrode type 10–23; Monokrystaly Turnov, Czech Republic) according to Houba et al. [33]. The N (mineral, ammonium, nitrate) content was calculated to the dry soil mass; the dry mass was determined gravimetrically (on laboratory scales) after drying of soil in the laboratory dryer at 105 °C to the constant weight [34].

The fridge-stored samples (at 4 °C) were used for soil respiration analyses. Soil respiration induced by citric acid (Cit-IR), D-trehalose (Tre-IR), N-acetyl-β-D-glucosamine (NAG-IR), L-arginine (Arg-IR) was measured using MicroResp® device (The James Hutton Institute, Scotland) and spectrophotometric measurement (Tecan Infinite 200 PRO; Tecan Trading AG, Switzerland) of chromogenic indicator (cresol red) for CO_2 emission in the form of agar-agar gel in the 96-well microplate [35]. Freeze-dried samples were used for N-acetyl-β-D-glucosaminidase (NAG) activity assay, based on the spectrophotometric measurement (Tecan Infinite 200 PRO) of the product (4-nitrophenol, PNP) in the reaction with PNP-derivate of the natural enzyme substrate [36].

2.3 Microbiologic and molecular biologic analyses

DNA extraction from freeze-dried samples was done to carry out microbiologic and molecular biologic analyses. DNA was extracted from 0.5 g of freeze-dried soil sample using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA). Isolated DNA was quantified using Nanodrop One (Thermo Scientific, USA) and used to determine the soil microbial abundance as well as diversity and composition of soil microbiome.

Abundance of soil microbiome

The SYBR-Green platform was used on a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, USA). Real-time PCR was performed to quantify genes amoA (coding for ammonium monooxygenase) to determine ammonia-oxidizing bacteria (AOB) and gene phaZ (coding for P3HB depolymerase) to determine generally all microbial P3HB degraders (phaZ) biomass in soil DNA extracts. The primers used were AMOA1F (5′ GGGGTTTC-TACTGGTGGT 3′) and AMOA2R (5′ CCCCTCKGSAAAGCCTTCTTC 3′) for AOB [37];

and PHBf (5′ CGTCTACCGCAACGGCACCAAGG 3′) and PHBr (5′ TGGGCGTAGTT-GCTGGCCGT 3′) for phaZ [38].

Diversity and composition of soil microbiome

The structure and composition of the prokaryotic and fungal communities in the substrates were analysed using high-throughput sequencing of fragments the prokaryotic 16S rRNA genes and fungal ITS regions. Specific regions of the rRNA genes of fungi ITS2 (18S) and bacteria V3–V5 (16S) were amplified by using primers F357 (5′-CCTACGGGAGGCAGCAG-3′) and R926 (5′-CCGYCAATTYMTTTRAGTTT-3′), or ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′- TCCTCCGCTTATTGATATGC-3′), respectively, with barcodes and the universal overhang. Illumina sequencing adaptors were introduced in the second PCR, all in accordance with the general instructions [39]. The products were evaluated by agarose electrophoresis, quantified with a fluorimetric, high sensitivity AccuGreen Quantification Kit (Biotium Inc., USA) and pooled into an amplicon library. Sequencing took place on a MiSeq unit (Illumina, USA) running the reagent kit v2 and paired-end 250 nt reads in an external laboratory (SEQme s.r.o., Czech Republic). After pairing and filtering, a total of 68,025,630 prokaryotic and 2,074,317 fungi reads were obtained and further processed.

2.4 Data processing and statistical analyses

The α-diversity of the procaryotic and fungal microbial communities was determined by the Simpson and Shannon diversity indexes while β-diversity by the Sørensen similarity index. While α-diversity is a measure of microbiome diversity applicable to a single variant, β-diversity is a measure of the similarity or dissimilarity of two communities [40].

Data processing and statistical analyses were performed using R, version 4.3.1. [41]. Canonical Correspondence Analysis (CCA) was carried out to provide advanced analysis of relation between taxonomical β-diversity and key soil nutrition properties and other available parameters. The X axis separated samples according to the added P3HB dose with low c(P3HB) on the left and high on the right. Y axes reflected the presence of maize in the soil with planted variants in the upper and unplanted in the lower part of the scatter plot. The scatter plot was divided into two subplots: one reflected the taxonomic groups (class level) and the other the soil factors (PAST 4.0) [42].

Principal Coordinate Analysis (PCoA) is multivariate eigen analysis method to explore and to visualize similarities or dissimilarities of data [43]. It converts data on distances between items into map-based visualization of those items, it also allows to identify groups or clusters. PCoA finds the main axes through a matrix and calculates a series of eigenvalues and eigenvectors. Each eigenvalue has an eigenvector, and there are as many eigenvectors and eigenvalues as there are rows in the initial matrix.

For characterization of the relationship among the treatments and selected soil properties, two-way analysis of variance (ANOVA) type I (sequential) sum of squares was used at significance level of 0.05 [44], where the first factor was c(P3HB) and the second factor was presence of maize (unplanted and maize-planted). The purpose of using two-way ANOVA was also testing the interaction between these factors. An effect size was measured using eta-squared (η_p^2) from package lsr [45]. To detect the exact difference among factor level means, it was used Tukey's honestly significant difference (HSD) test from package agricolae [46] and treatment contrast for calculating factor level means with standard error of mean (SEM). These results were graphically represented with bar charts showing statistically significant difference at significance level of 0.05 indicated by different letters. Lowercase letters indicate differences among c(P3HB), uppercase letters between unplanted and maize-planted variants with dependence of P3HB dose.

The data on diversity and composition of soil microbiome were further processed with the DADA2 v1.26.0 R package [47] with help of MetaCentrum high performance computing service and visualized by the phyloseq v1.42.0 R package [48] and ComplexHeatmap v2.14.0 R package [49]. Package ggplot2 v3.5.1 [50] was used for creating advanced statistical graphs.

Microbiome taxonomy was assigned for the bacteria according to the SILVA 132 SSU NR 99 reference database [51] and the 8.3 release of the UNITE reference database for fungi [52].

3. Results

3.1 Microbial diversity response to P3HB and maize

Despite the differences in relative abundances (RAs) of prokaryotes and fungi (Fig. S1), the Shannon and Simpson indexes showed no significant differences between the prokaryotic communities of the variants (Fig. S2a, b). Similarly, there were no significant differences in fungal communities' diversity in the maize-planted variants (Fig. S2c, d). In contrast, the unplanted variants indicated an effect of P3HB on fungal α-diversity – the Shannon index significantly decreased at 1% c(P3HB) and remained at a similar level for higher c(P3HB) (Fig. S2c); the Simpson index exhibited a deviation but without a trend (Fig. S2d).

PCoA (Fig. S3a) of the prokaryotic community revealed no effect of maize on β-diversity, however, a visible effect of P3HB starting at around 5% c(P3HB) (Fig. S3a). Fungal β-diversity PCoA revealed a noticeable effect of the plant presence and markable separation of control and low c(P3HB) variants from moderate to high ones (Fig. S3b).

3.2 Soil taxonomic groups response to P3HB and maize

The changes in soil microbiome composition were comprehensively documented by the CCA plots (Fig. 1) and changes in RA levels of the taxonomical group's representatives expressed as percentage of all the identified operational taxonomic units found by sequencing.

At the family level, for example, *Nitrososphaeraceae* and *Vicinamibacteriaceae* were negatively influenced by increasing c(P3HB) in the prokaryotic community, while other families, such as *Microbacteriaceae, Caulobacteriaceae, Pseudomonadaceae, Rhizobiaceae*, reacted by the expansion of their presence (Fig. S1c). Fungal families *Helotiaceae*, *Herpotrichiellaceae* and *Pseudeurotiaceae* were stimulated by increasing c(P3HB); other fungal families, especially *Nectriaceae* and *Plectosphaerellaceae*, were rather suppressed (Fig. S1d). The effect of maize was mostly insignificant (Fig. S1a, b).

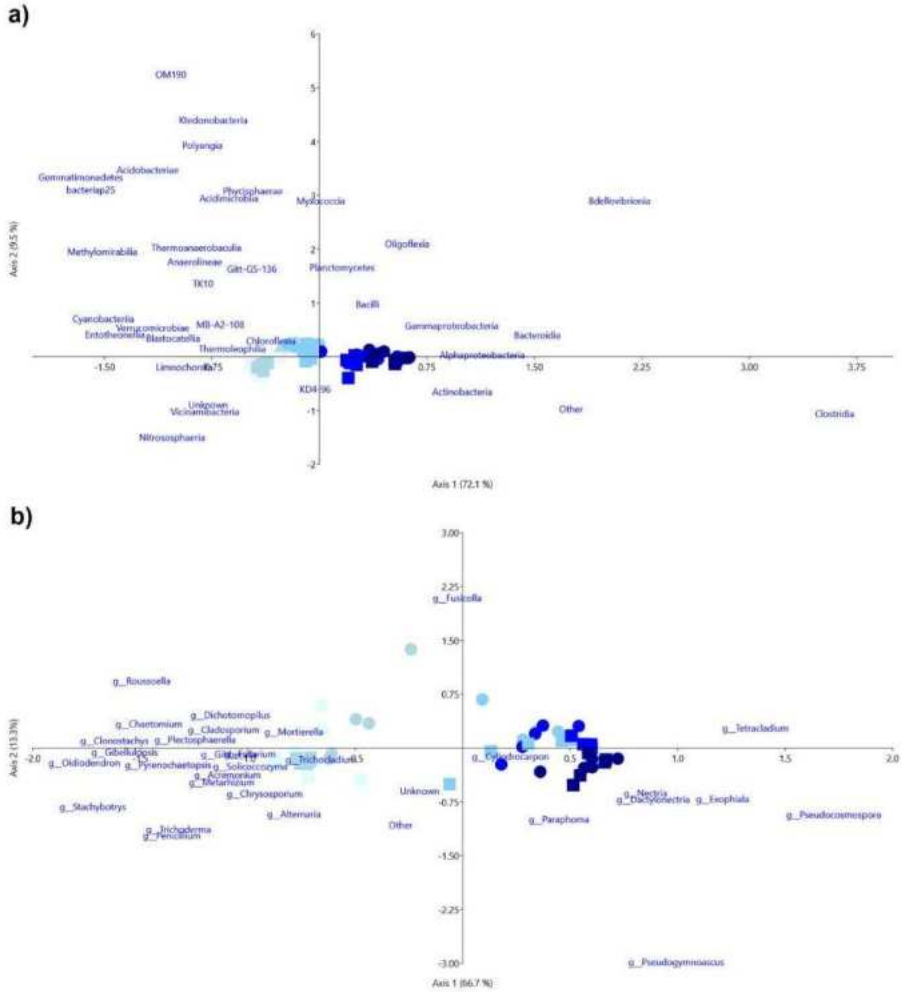

Fig. 1 Canonical correspondence analysis of microbial community composition. Scatter plots showing relations of (a) prokaryotic community at the class level and (b) fungal community at the genus level in unplanted (square) or planted (circle) soil variants contaminated with variable doses of P3HB (0, ● 0.1, ● 1, ● 5, ● 10%). Related statistical data are in Tables S1 and S2

The prokaryotic microbiome analysis showed that *Bacteria* generally dominated over *Archaea*, which were represented mostly by microorganisms involved in nitrification (Fig. S4a). This natural domination was graded in favour of *Bacteria* with increasing c(P3HB) as RAs of *Archaea* decreased in both unplanted and planted soil (Fig. S4b).

Enrichment of the prokaryotic community with *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* and anaerobes *Clostridia* was found for high c(P3HB) variants (Fig. 1a). Control and low c(P3HB) variants were characterized by higher abundance of *Nitrososphaeria*, *Nitrospiria*, *Thermoleophilia* and *Vicinamibacteria* (Fig. 1a).

The CCA plots of both bacterial and fungal taxa (Fig. 1a, b) showed markable dispersion of variants along X axis indicating a determinative influence of P3HB and strict separation of 0% + 0.1% and 1–10% variants, while the influence of maize was weaker than on the prokaryotic community (Fig. 1a). *Tetracladium*, *Exophiala, Pseudogymnoascus, Pseudocosmospora* and *Nectria* were stimulated by high c(P3HB); these taxa overlaid all other fungi taxa (Fig. 1b).

Actinobacteria, Alphaproteobacteria and *Gammaproteobacteria* represent mostly copiotrophic taxa. RAs of these prokaryotic groups showed growth following P3HB contamination (Fig. S6a, Fig. 2). *Actinobacteria* was the most abundant prokaryotic group, stimulated by P3HB in both planted and mainly unplanted soil with the highest RA reaching 30% in 10% P3HB maize planted variant (Fig. 2a). RA of *Alphaproteobacteria* was even more directly dependent on c(P3HB) in the soil (Fig. 2b). RA of *Gammaproteobacteria* mostly significantly increased at 1–10% c(P3HB) in both unplanted and planted soils without another trend (Fig. 2c).

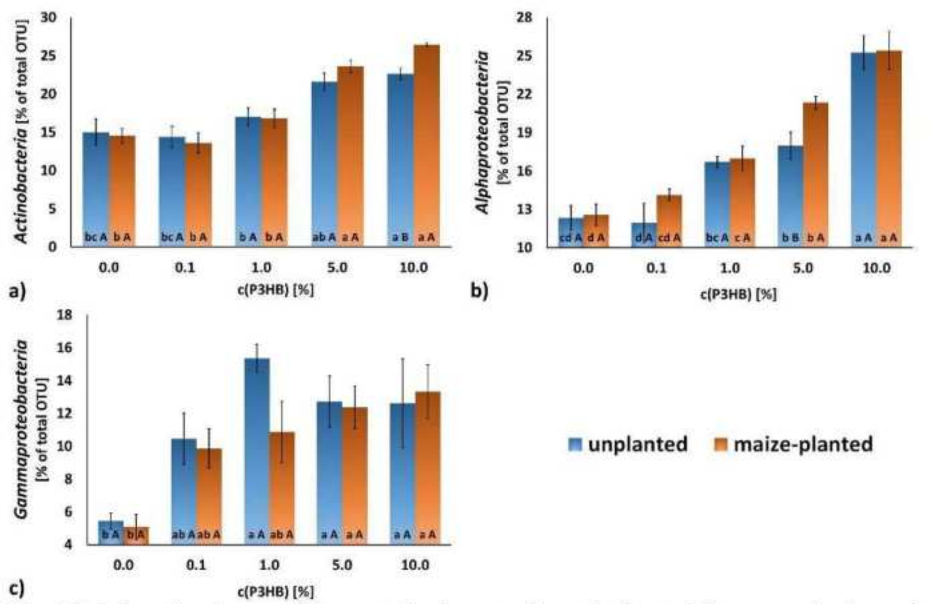

Fig. 2 Relative abundances of the most dominant prokaryotic (bacterial) taxonomic classes in the variants. Average values ($n = 5$) with standard errors of mean (error bars); lowercase letters indicate differences between all the variants (evaluated separately for unplanted and planted variants), uppercase letters between unplanted and planted variant of respective P3HB dose

On the contrary, RA of oligotrophic *Vicinamibacteria*, the fourth most abundant prokaryotic group (Fig. S6a), decreased with increasing c(P3HB) in both unplanted and planted soil (Fig. S5a). Similar trend showed oligotrophic bacteria class *Thermoleophilia* (Fig. S5b).

The RA of groups strongly involved in N cycling, i.e. nitrifying prokaryotic classes *Nitrososphaeria* belonging to *Archaea* and *Nitrospiria*, which oxidize nitrites to nitrates, were inversely related to c(P3HB) both in unplanted and planted soil (Fig. 3a,b), following the *Archaea* results (Fig. S4b). However, slightly steeper decrease in the RAs of the classes was found in planted soil.

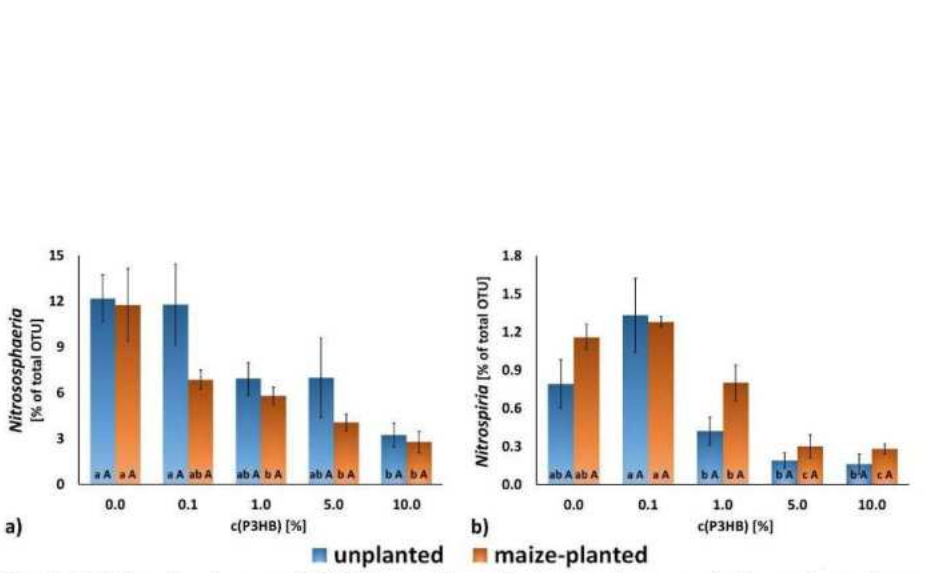

Fig. 3 Relative abundances of nitrifying prokaryotic taxonomic groups in the variants. Average values (n = 5) with standard errors of mean (error bars); lowercase letters indicate differences between all the variants (evaluated separately for unplanted and planted variants), uppercase letters between unplanted and planted variant of respective P3HB dose

Another significantly P3HB-affected (Fig. 1a, Fig. S6a) class was anaerobic *Clostridia*. Its RA increased significantly at the high c(P3HB) (Fig. S5c), indicating more anaerobic environment in both unplanted and planted soil of the respective variants.

P3HB-stimulated fungal genera (Fig. 1b, 4a–c, S7) *Tetracladium*, *Exophiala, Pseudogymnoascus, Pseudocosmospora* and *Nectria* began to form a dominant fungal group at the high c(P3HB) (S6b). *Tetracladium* became dominating taxa starting at 1% c(P3HB) in both unplanted and planted soil. *Exophiala* had a similar progressive but more gradual development (Fig. 4b). *Pseudogymnoascus* reacted at 10% c(P3HB) in both unplanted and planted variants (Fig. 4c). On the contrary, abundance of *Gibberella* and *Gibellulopsis* as well as other taxa were affected negatively by c(P3HB) (Fig. 4d,e, S6b).

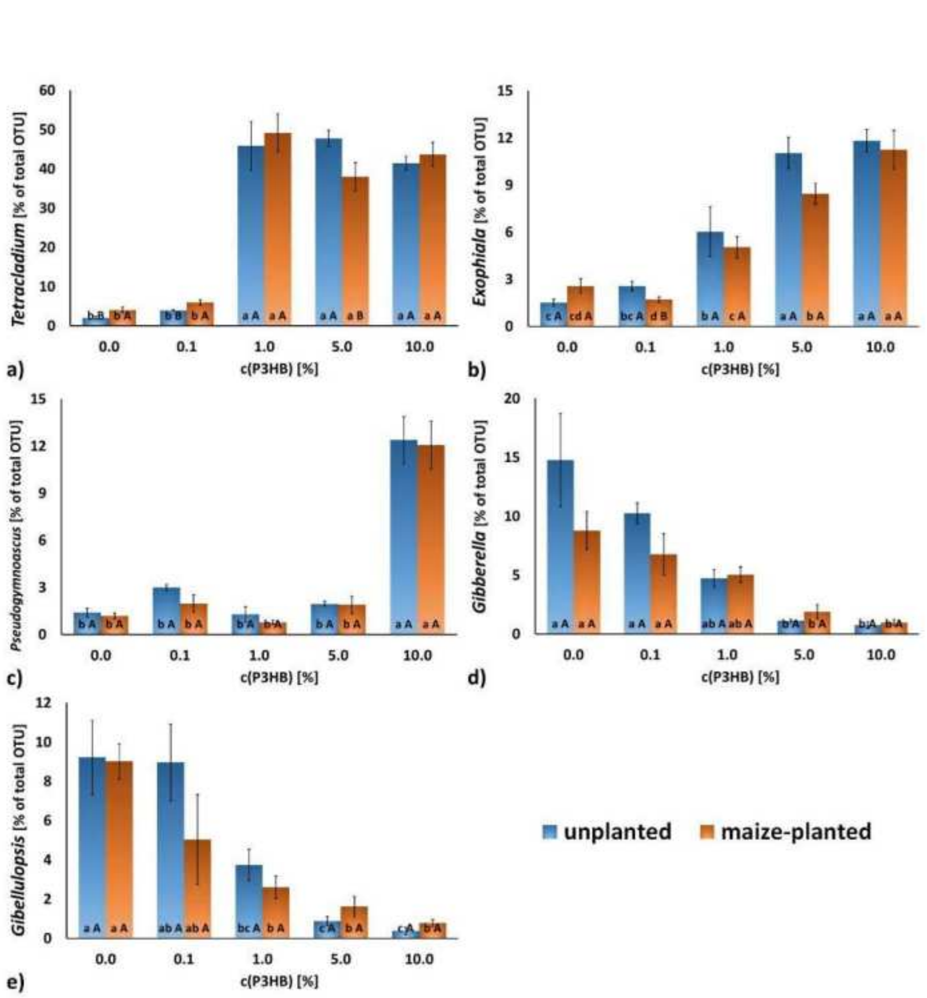

Fig. 4 Relative abundances of fungal taxonomic groups in the variants. Average values ($n = 5$) with standard errors of mean (error bars); lowercase letters indicate differences between all the variants (evaluated separately for unplanted and planted variants), uppercase letters between unplanted and planted variant of respective P3HB dose

3.3 Microbial activity and biomass response to P3HB and maize

All the analysed substrate-induced respirations (IRs) and NAG showed stimulation by increasing c(P3HB) in both unplanted and planted soil (Fig. 5). The growth of IR values was generally more intense at high c(P3HB) (Fig. 5a–d); NAG showed similar results (Fig. 5e). The presence of maize was significant for all the characteristics, but with an ambiguous trend.

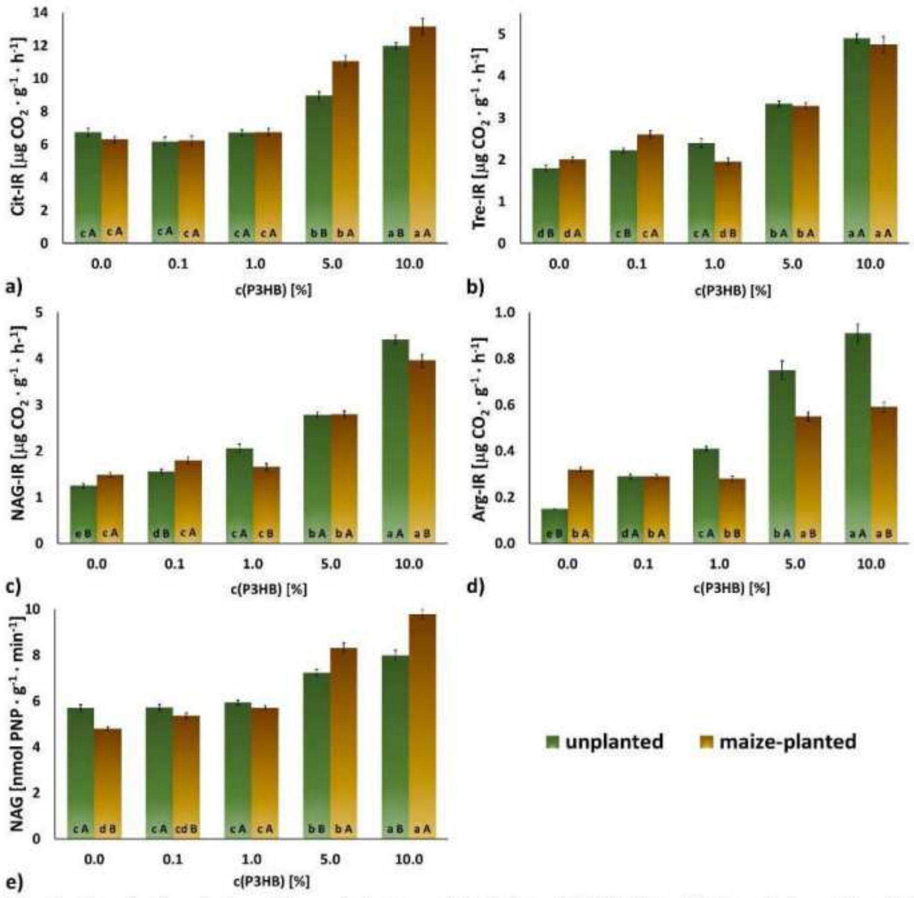

Fig. 5 Respiration induced by substrates – (a) citric acid (Cit-IR), (b) D-trehalose (Tre-IR), (c) N-acetyl-β-D-glucosamine (NAG-IR), (d) L-arginine (Arg-IR) – and (e) N-acetyl-β-D-glucosaminidase (NAG) enzyme activity in the variants. Average values (n = 5) with standard errors of mean (error bars); lowercase letters indicate differences between all the variants (evaluated separately for unplanted and planted variants), uppercase letters between unplanted and planted variant of respective P3HB dose

The phaZ was clearly stimulated by high P3HB doses in both planted and unplanted soil (Fig. 6a). The positive effect of maize at 0–0.1% c(P3HB) disappeared with increasing c(P3HB).

Biomass of AOB indicated the decisive influence of maize (Fig. 6b). While in the unplanted soil the increasing c(P3HB) rather led to a decrease in AOB, in the planted soil c(P3HB) ≥1% led to its stimulation. However, the differences were minor.

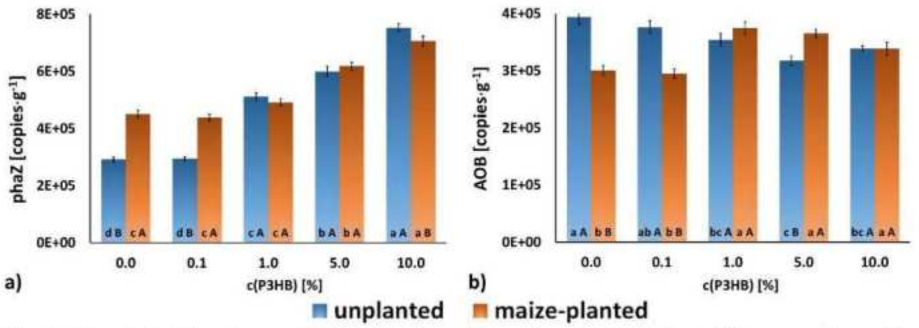

Fig. 6 Microbial abundance of (a) P3HB-degrading microbes (phaZ) and (b) ammonia-oxidizing bacteria (AOB) in the variants. Average values (n = 5) with standard errors of mean (error bars); lowercase letters indicate differences between all the variants (evaluated separately for unplanted and planted variants), uppercase letters between unplanted and planted variant of respective P3HB dose

3.4 Soil N response to P3HB and maize

The NH_4-N content in the unplanted soil showed a Gaussian function-shaped trend with maximum value at 1% P3HB (Fig. 7b). NH_4-N content in the planted soil showed positive direct dependence on the increasing c(P3HB) with the only exception in the 5% P3HB variant (Fig. 7b). The presence of maize was a significant factor in all variants.

In the case of NO_3-N and N_{min}, the presence of maize was also a significant factor. While in the unplanted soil both NO_3-N and N_{min} decreased with increasing c(P3HB), weaker and mostly indirect dependence was visible in the presence of maize (Fig. 7c).

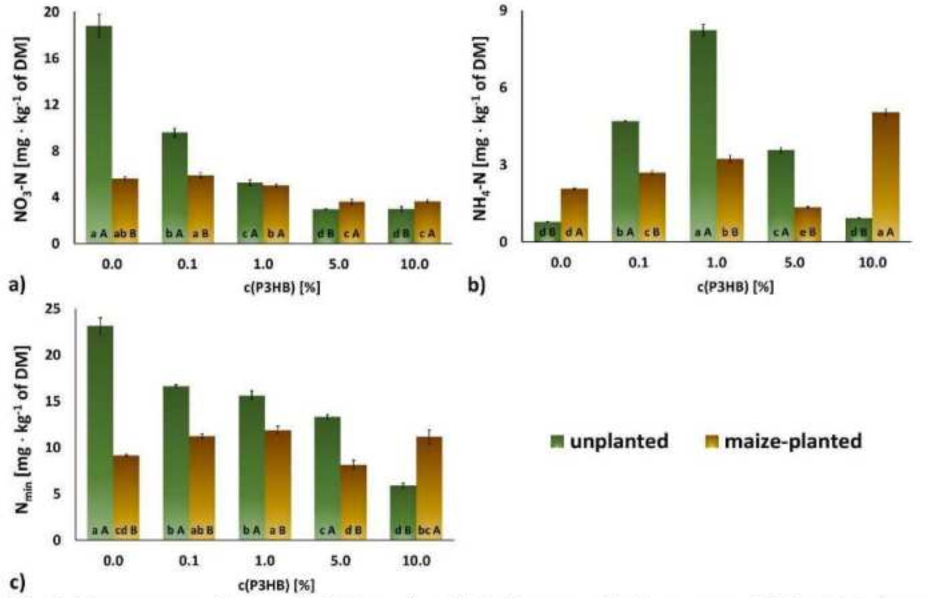

Fig. 7 The content of inorganic N forms in soil. Soil content (in dry matter = DM) of (a) nitrate nitrogen (NO_3-N), (b) ammonium nitrogen (NH_4-N) and (c) mineral nitrogen (N_{min}) in the variants. Average values (n = 5) with standard errors of mean (error bars); lowercase letters indicate differences between all the variants (evaluated separately for unplanted and planted variants), uppercase letters between unplanted and planted variant of respective P3HB dose

3.5 Effect of P3HB on plant growth

AGB of maize significantly decreased reaching moderate c(P3HB) in soil (Fig. S9). This content was decisive for suppressing the growth of maize; a further increase in c(P3HB) could not have any further negative effect on biomass production. The decisive effect of P3HB and related microbial activity in the soil suppressing the growth of maize was also highlighted by the CCA plots with clear grouping of (I) separated AGB, (II) NO_3-N and (III) mostly clustered remaining characteristics (Fig. S8).

4. Discussion

4.1 Changes in soil microbial community diversity

The unchanged α-diversity of *Prokaryota* (Fig. S2a, b) does not align with previous results. In soils contaminated with BPs, some authors [8, 53] observed an increase, whereas other studies [54] reported a decrease. This contrasts with β-diversity, which continuously shifted in response to the c(P3HB) gradient (Fig. S3a).

Fungal community diversity was affected by P3HB, as its Shannon index α-diversity decreased at 1% c(P3HB) in unplanted soil (Fig. S2c). Additionally, β-diversity was strongly influenced by both P3HB and the presence of maize (Fig. S3b). In the presence of maize, the Shannon index α-diversity followed the same trend, though the differences were not statistically significant. The stronger fungal response (Fig. 1), evident from the suppression of certain fungal genera compared to prokaryotes, was also observed in the RA of taxonomic groups (Fig. S6b). While most fungal genera were suppressed at moderate c(P3HB) levels, the critical effect on *Prokaryota* occurred starting at 5% c(P3HB). These results indicate a higher sensitivity of fungi to P3HB addition and confirm first hypotheses that increasing c(P3HB) in soil modifies microbial fungal community towards lower diversity.

4.2 Changes in soil microbial community composition

Both prokaryotic and fungal community compositions were affected by the presence of P3HB in soil (Fig. 1, 2, 3, 4 S4, S5, S6). The dominance of bacterial RAs compared to *Archaea* increased with higher c(P3HB) (Fig. S4), as the RA of *Archaea* decreased (Fig. 2a). Despite their ability to synthesize and metabolize PHAs [55], soil *Archaea* are more involved in nitrification [56], a process heavily altered by P3HB contamination.

Highly P3HB-stimulated copiotrophic classes *Actinobacteria, Alphaproteobacteria* and *Gammaproteobacteria* (Fig. 2, S6a) probably included most of prokaryotic P3HB degraders (phaZ gene) in this experiment (Fig. 1a; 6a). The ability of *Actinobacteria* to degrade P3HB is well-known [57] and often coupled with the potential of P3HB synthesis and accumulation [58]. The *Alphaproteobacteria* class also includes some representatives of PHA degraders [59, 60]. These originally common taxa likely switched to a preferentially utilizing P3HB as a C and energy source, accompanied by progressive *Gammaproteobacteria*.

Increasing c(P3HB) led to the formation of a dominant fungal group consisting of the genera *Tetracladium*, *Exophiala* and *Pseudogymnoascus*, with weak enrichment in other known and unassigned fungi (Fig. S6b). This finding confirms the existence of an abundant P3HB-degrading fungal population in the soil. The increased aerobic mineralization of C sources (Fig. 5b–d), representing products of fungal growth, metabolism, and biomass turnover, indicated a strong fungal involvement in P3HB degradation. The booming *Tetracladium* from *Ascomycota* group might be the most actively involved.

Tetracladium species, such as *Tetracladium marchalianum,* can degrade carboxymethyl-cellulose, xylan and polygalacturonic acid [61]. *Tetracladium* genomes were found to contain various esterases, lipases and pectate lyases, which are important enzymes for the degradation of some polyesters e.g., poly(butylene succinate-co-adipate) (PBSA) [62]. The authors of the aforementioned study reported that fungal communities on PBSA and in the surrounding soil were strongly dominated by *Tetracladium* spp. (RA 42−45%), and the PBSA micro-BPs were substantially mineralized to CO_2. Our results (RA up to 50%; Fig. 4a, S6b) are consistent with these findings.

Toxic and extreme-tolerant class *Exophiala,* belonging to the black yeast *Ascomycota* group, also thrived at 1−10% c(P3HB) (Fig. 4b, S6b). Black yeast species, such as *Exophiala oligosperma* R1, *E. xenobiotica* or *E. jeanselmei*, have been shown to degrade various C substrates, e.g., aromatic hydrocarbons or low-molecular-weight urethane compounds [63-66]. Their abilities predispose them to be efficient in BP degradation and support our findings.

The psychrotolerant *Ascomycota Pseudogymnoascus* responded positively to the highest P3HB contamination (Fig. 4c, S6b). *Pseudogymnoascus* species are known to be cellulolytic and function as saprotrophs [67]; they are either psychrophilic or psychrotolerant and abundant in soils with high fertility [68] indicated here by high c(P3HB) and originally fertile arable soil. The involvement of their representatives in BPs degradation was found recently [69].

The negative effects of P3HB on the soil microbiome and the decrease in RAs of groups such as *Thermoleophilia, Vicinamibacteria, Nitrospiria, Nitrososphaeria*, *Gibberella* and *Gibellulopsis* (Fig. 3, 4d,e, S5, S6) may be associated with related changes in elemental cycles and metabolic conditions for these microorganisms. Thus, the second hypothesis was confirmed.

4.3 Changes in soil microbial behaviour and processes related to C

The CCA results (Fig. 1, S8) provided further evidence of changes in the composition and behaviour of the microbial community and the association of P3HB degraders with the mentioned stimulated groups of bacteria and fungi. These changes were accompanied by a shift in the substrate consumption preferences from intrinsic soil organic C to P3HB [21, 23], as confirmed by the increase in both the phaZ gene (Fig. 6a) and microbial activity properties (Fig. 5). This shift probably initiated further changes in soil microbial activity, metabolic processes and soil environment.

The results are of particular interest as they do not align with the general understanding of the adaptation of fungi and bacteria to shifts in SOM (i.e. substrate) quality. From a metabolic pathway perspective, bacteria often possess more simplified, direct, and diverse metabolic pathways compared to fungi, enabling faster adaptation to various conditions and stresses. Bacteria produce a wide range of enzymes that allow them to quickly metabolize various substrates quickly, whereas fungi typically have more complex metabolic pathways and rely on extracellular enzymes to break down complex organic molecules. Bacteria reproduce rapidly and can quickly colonize and adapt to new environments, although their growth is often limited by the availability of nutrients. On the contrary, fungal growth and adaptation to stressors are generally slower.

4.4 Changes in soil microbial behaviour and processes related to oxygen (O)

All the above-discussed leading stimulated taxa represent aerobic microorganisms, while the *Clostridia* class also includes anaerobic species. *Clostridia* proliferation was highly stimulated at high c(P3HB) levels (Fig S4c); this may indicate a decrease or depletion of O_2 in the soil following the strongly enhanced P3HB mineralization-based respiration (Fig. 5). This factor could have contributed to the deterioration of environmental conditions for other soil organisms. Furthermore, a decrease in O_2 in the vicinity of plant roots could exacerbate the adverse effect of P3HB on plant roots [70]. To support or reject this new hypothesis, a pilot respiration/biodegradation experiment was conducted (Text S1).

The experiment revealed a sharp decrease in O_2 in initial stage of biodegradation (Text S2). After a few weeks, the O_2 level increased again stabilized although it was still slightly lower compared to the control. That implies that O_2 concentration fluctuates during the biodegradation process and its consumption for biodegradation is higher in the initial stage of the process. As a result, we conclude that for plant growth in the presence of biodegrading P3HB, the O_2 shortage may be problematic mainly in the early stage of P3HB introduction, where is the rate of biodegradation highest (Text S2). If the initial phase of plant growth occurs simultaneously as in this and other experiments [21, 23], this deficiency can be critical. This aligns to results of Brown et al. [5], who have analysed stress markers in maize grown in the soil amended with PHBV microplastics. Even at low PHBV concentrations, the authors found significantly higher content of lactic acid (a product of anaerobic respiration), which authors attributed to a response of plant to PHBV-induced hypoxia in the soil.

From the environmental perspective, under strictly anaerobic conditions (e.g. in paddy soils), the enhanced proliferation of anaerobic microorganisms is problematic due to possible proliferation of methanogenic microorganisms and the subsequent production of methane [71], which is a common product of ultimate biodegradation under anaerobic conditions. Under common conditions, ideal for P3HB biodegradation, the level of O_2 may vary, but the conditions may not be supportive for methanogenesis. Nevertheless, as shown by Lussich et al. [72] or discussed by Schlüter et al. [73], due to local O_2 shortage in otherwise well-aerated soils, microbial activity in hotspots with easily degradable organic compounds and available nitrate (e.g. arable soils) may facilitate denitrification leading to N_2O emissions [74]. Hence, the additional research is needed to shed light on this environmental aspect of P3HB and other BPs biodegradation.

4.5 Changes in soil N mineralization

N mineralization is a process by which microorganisms in the soil decompose organic N compounds into inorganic forms, primarily ammonium (NH_4^+). This process occurs as part of the decomposition of organic matter and added substrates. It involves several consecutive steps such as decomposition (microbial breakdown of organic matter), ammonification (conversion of the organic N into ammonium) and, in well-aerated soil, an optional step of nitrification (conversion of ammonium into nitrate NO_3- by nitrifying bacteria) [75]. In line with above-discussion, we speculate that the P3HB-induced decrease of AOB in the unplanted soil (Fig. 6b) may be related to the decrease in the RA of *Archaea* (Fig. S4). AOB and ammonia oxidizing *Archaea* occupy the same ecologic niche, differentiated only by the availability of ammonium in the environment [55, 76]. Ammonia-oxidizing *Archaea,* including *Nitrososphaeria*, are abundant in warm and humid soils, along with AOB (including *Nitrospiria*). Both groups play a significant role in soil nitrification. *Nitrososphaeria* represents a dominant group of ammonia-oxidizing *Archaea* within *Nitrososphaerota* in arable soils [53], while *Nitrospiria* is the key bacterial group for soil nitrification [77]. Therefore, the decreasing RAs of both classes with increasing c(P3HB) (Fig. 3) confirmed changes in N cycling in the soil, which were directly reflected by altered contents of N forms (Fig. 7).

The decrease in the RA of *Archaea* with increasing c(P3HB) can be explained by the addition of a substrate that does not contain N or NH_2 groups. In other words, there is no N-mineralization, and nitrifying organisms are not needed as ammonia or ammonium ions are not released. Instead, other types of organisms proliferate and exploit these new conditions. A predominant decrease of NO_3-N, N_{min} (Fig. 7a,c) and AOB (Fig. 6b) with increasing c(P3HB) in the unplanted soil supported the assumption of a strong negative effect of soil P3HB contamination on nitrification. This effect was modified by the presence of maize, mostly due to competition between the plant and microbes for N acquisition. N uptake by maize decreased NO_3-N and N_{min} in the control (Fig. 7a,c), however, the difference gradually decreased and even reversed with increasing c(P3HB), which may be related to the severely limited plant growth starting at 1% c(P3HB) (Fig. S9).

The preferential boosted utilization of high c(P3HB) as the primary C source in soil, accompanied by increased utilization of N compounds from SOM (Fig. 5d, 6a), enhanced nutrient uptake and catabolism. This likely resulted in a partially anoxic environment (chapter 4.4) with a more severe impact in planted soil due to the parallel root respiration exhausting soil O_2. Partial anaerobiosis could lead to the subsequently inhibited nitrification (Fig. 3) and explain NO_3-N depletion (Fig. 7a), as nitrate could be used as an alternative electron acceptor for P3HB degradation [78]. The same mechanism can explain higher NH_4-N content in the moderate unplanted P3HB variant and rather increasing NH_4-N content in the planted soil (Fig. 7b) because nitrification is prevented by O_2 limitation. As a result, 1% c(P3HB) in the soils (used in this work or similar in terms of SOM content) appears to be a critical breakpoint above which crucial changes occur.

Therefore, the third hypothesis was confirmed. Furthermore, we can assume that some microbial groups also responded to changes in soil N availability.

4.6 Plant response to P3HB

The combined impact of P3HB-derived changes in all the studied soil parameters, including shifted microbial community composition, diversity changes, increased C mineralization interfering with nitrification and decrease in inorganic N as well as O_2 availability (Fig. S8), resulted in the strongly suppressed maize growth starting at medium c(P3HB) (Fig. S9). NO_3-N availability was assumed as the main limiting factor; however, the role of soil aeration seems to be also significant. To determine the decisive factor, future studies should focus on monitoring over shorter time periods as the preliminary results show that the first 2–3 weeks can be critical (Text S2).

5. Conclusions

The contamination of soil with P3HB microplastics induced significant changes in the composition and functions of microbial communities following enhanced C mineralization. Procaryotic α-diversity was not affected by P3HB, whereas fungal α-diversity declined starting at medium P3HB levels. The composition of prokaryotic and fungal communities shifted from a balanced microbiome, including oligotrophic and nitrifying groups, towards prevailing copiotrophic taxa, with a sharper shift in the eucaryotic community. This shift enhanced respiratory mineralization of P3HB-contained C and likely led to local O_2 depletion, promoting the abundance of anaerobes impacting N mineralization. The decrease in nitrifying taxa limited the availability of mineral N in soil, particularly nitrates, due to the reduced oxidation of ammonia and possible anaerobic N respiration. Medium and high P3HB doses probably exceeded a critical threshold, triggering a transition in microbial community composition and dependent nutrient transformation from nutrient sequestration and availability to over-enhanced nutrient consumption, followed by the inhibition of plant growth.

A general recommendation can be derived for the safe limits of P3HB-based material use in agriculture. The critical effects started at approximately 1% of P3HB microplastics in soil. Therefore, for example, the application of 100 μm thick P3HB film, considering its decomposition and mixing with topsoil (10 cm), will result in P3HB content of about 0.1%, which corresponds to a safe dose. However, repeated applications of P3HB without allowing adequate time for its complete biodegradation between applications can increase its soil content to levels greater than or equal to 1%. Such accumulation may negatively impact soil quality and fertility over time.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: T.H., J.H., J.K. and M.B.; Data curation: T.H., J.S.; Formal analysis: T.B., A.K., J.S.; Funding acquisition: M.B., J.K. and M.K.; Investigation: T.H., J.H. and M.B.; Methodology: T.H., J.H. and M.B.; Project administration: M.B.; Resources: J.H., T.H., J.S. and A.K.; Software: T.B., T.H. and J.H.; Supervision: V.P., J.K., M.K.; Validation: V.P., J.K., M.K., A.K., M.B.; Visualization: T.H., J.H., M.K. and V.P.; Writing - original draft: J.H., M.B.; Writing - review & editing: V.P., J.K., M.K.

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