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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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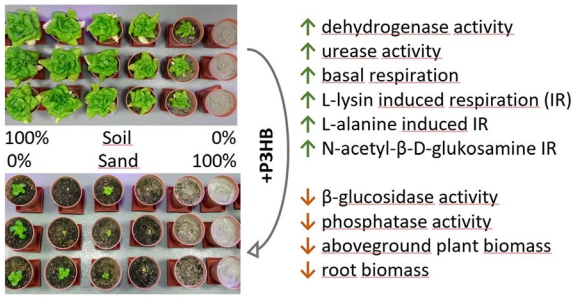
**Graphical Abstract**




## 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

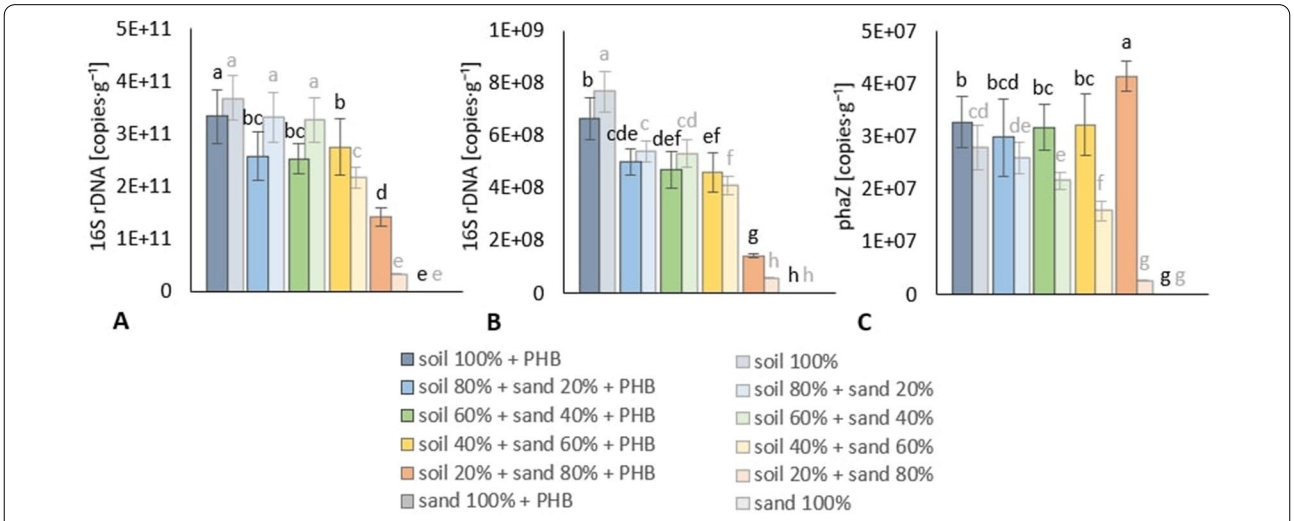



**Fig. 1** **A** Bacterial (16S rDNA), **B** fungal (18S rDNA), **C** PHB-degrading microorganisms (*phaZ*) gene markers in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

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

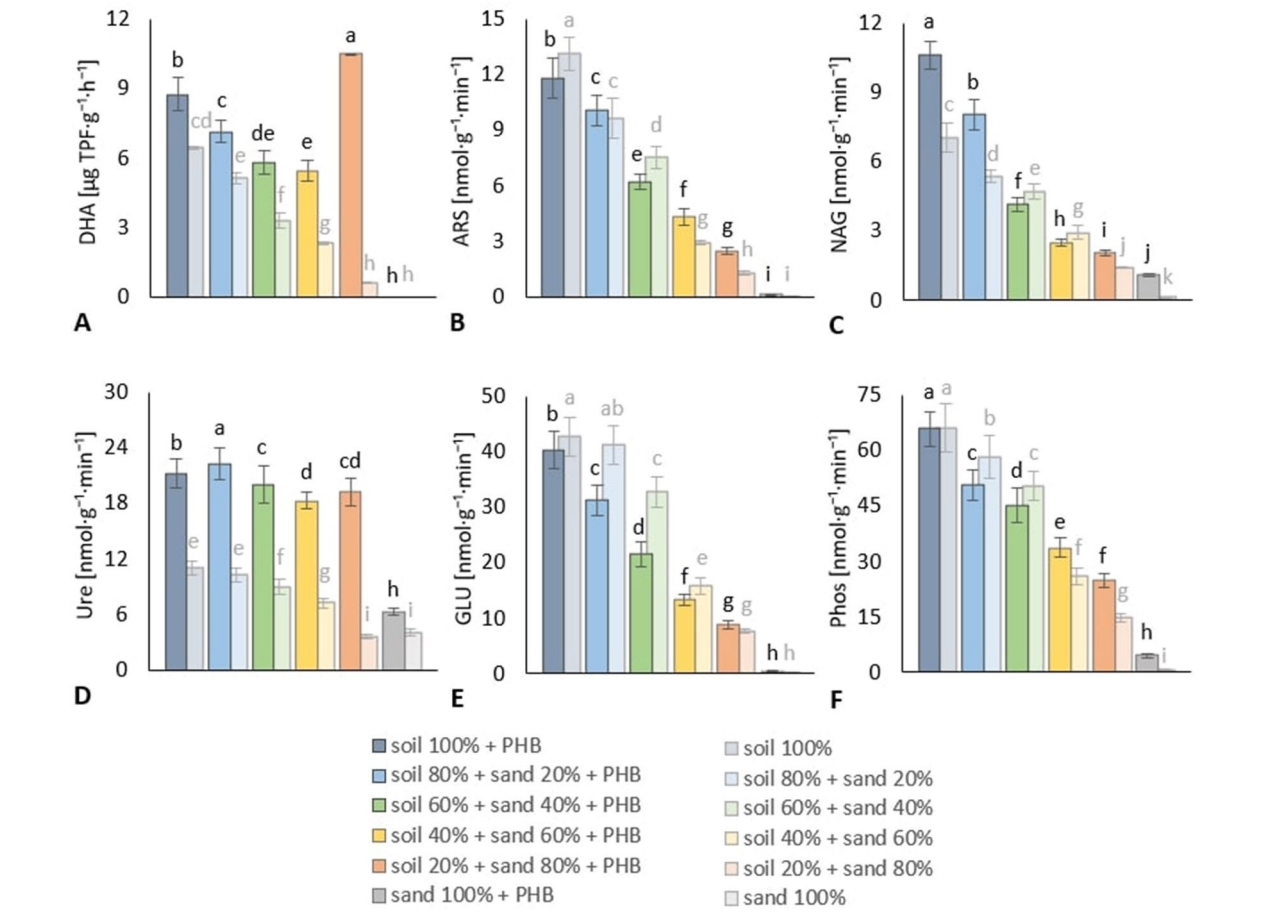



**Fig. 2** Soil activities of **A** dehydrogenase activity, **B** arylsulfatase, **C** N-acetyl-β-D-glucosaminidase, **D** urease, **E** β-glucosidase and **F** phosphatase in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

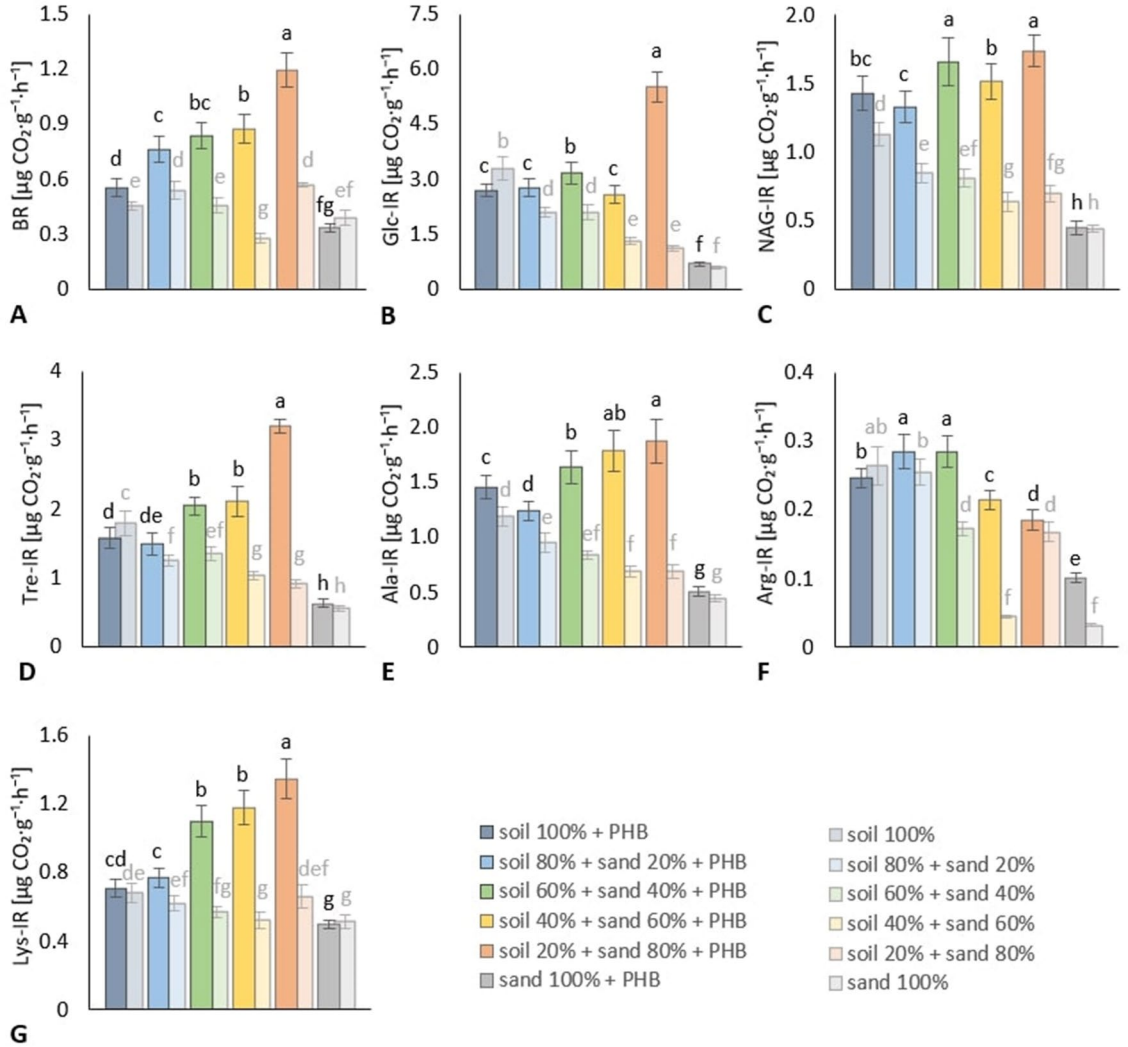



**Fig. 3** **A** Basal, **B** D-glucose, **C** N-acetyl-β-D-glucosamine, **D** D-trehalose, **E** L-alanine, **F** L-arginine and **G** L-lysine induced respiration in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

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

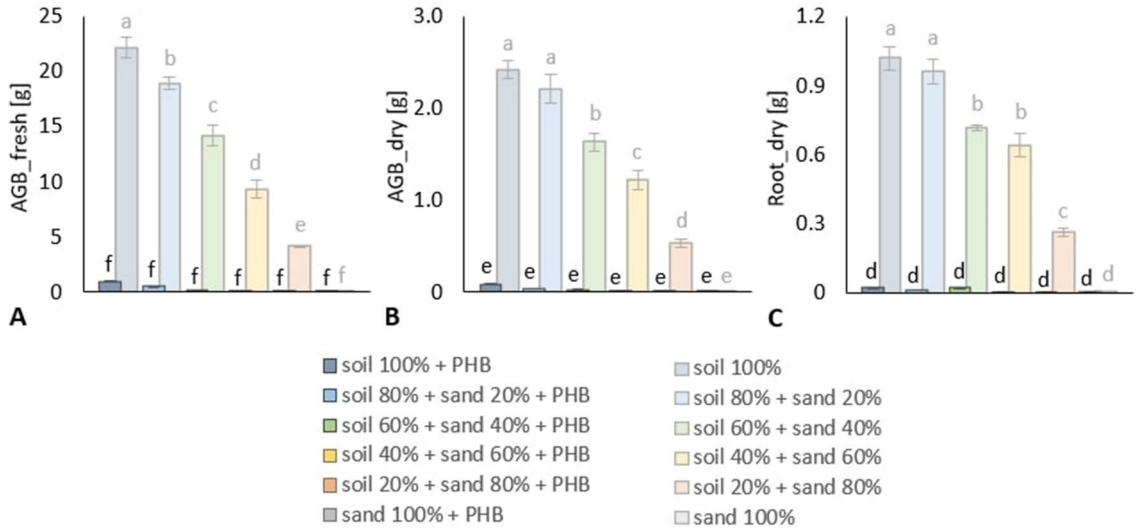



**Fig. 4** **A** Fresh and **B** dry aboveground plant biomass (AGB) and **C** dry root plant biomass in the growth substrates; the lowercase letters indicate significant differences at $p \leq 0.05$

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 (≥ 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

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

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