After Life Effect of Endophytic Bacterium *Bacillus Cereus* on Litter Decomposition

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Abstract

Background and aims: Endophytic microorganisms exist commonly in plants and are recognized to increase plant growth especially under adverse physical environmental conditions. We here demonstrate that endophytic bacteria (EB) *Bacillus cereus* can accelerate the decomposition of plant litter and enhance nutrient availability for plant growth.

Methods: We first obtained plant litter with and without EB inoculation using a model plant *Arabidopsis thaliana* in a microcosmic experiment, then conducted a litter decomposition experiment to investigated the effect of EB on litter decay rate, phosphorus availability, and on soil microbial community structure. We further evaluated wheat (*Triticum aestivum*) biomass growth using soils treated with and without EB.

Results: Inoculation of EB significantly increased the mass loss of *Arabidopsis* litter in the middle stage of decomposition, elevated the activity of alkaline phosphatase in the early stage of decomposition, and increased soil available P at the end of decomposition. Analyses of Illumina MiSeq sequencing and structural equation models also indicated that EB inoculation had pronounced impact on the bacterial abundance and diversity in soil. Finally, the growth of the wheat was significantly promoted in the litter with EB decomposition system.

Conclusion: EB mediated host after-life effect likely through accelerating the release of nutrients such as P from decomposing plant litter and regulating the structure of soil microorganisms, promoting the sustainability of nutrient utilization efficiency in a terrestrial ecosystem.

1. Introduction

Plant litter represents a major source of organic matter, and its decomposition plays an important role in carbon storage and nutrient cycles, acting as a regulator in most terrestrial ecosystems (Aerts and Chapin 1999; Berg and McClaugherty 2014). Besides, plants also have an important “after-life” effect (or imprint) on ecosystem processes, which can in turn feedback to determine net primary productivity (Knops et al., 2001). Many studies have shown that the functional diversity and activity of soil microorganisms can influence rate of litter decomposition (Chapman et al. 2013; Frainer et al. 2015; Hines et al. 2016; García-Palacios et al. 2016). Soil microbes play an important role connecting plant growth and soil mineral nutrition (Tang et al. 2014; Faucon et al. 2015; Wu et al. 2018). It is estimated that microbes are responsible for up to 90% of organic matter decomposition (Persson et al. 1980; Gołębiewski et al. 2019; Ramirez et al. 2012).

Many studies have shown that microbial community turnover can have important impacts on ecosystem processes such as litter decomposition (Strickland et al. 2009; Ramirez et al. 2012; Wu et al. 2019). An increasing number of studies has reported that decomposition rates match measures of metabolic activity and microbial abundance (Santschi et al. 2018; Rossi et al. 2018; Wu et al. 2019). The relative abundance of bacteria vs. fungi (indicated by fungi-to-bacteria ratios) and functional groups of decomposers will be successional during the litter decomposition process (Torres et al. 2005; Chapman et

al. 2013). Reduced functional and trophic complexity in microbial communities had been found to reduce the rate of decomposition (Santschi et al. 2018). Although soil microbial diversity has been well studied, the factors that modulate its structure are still relatively unclear. Research on the influence of microbial succession on litter decomposition has focused predominantly on different vegetation (Chapman et al. 2013; Wu et al. 2019), climate change (Butenschoen et al. 2011; Hines et al. 2016) and litter chemical composition (Santonja et al. 2017; Santschi et al. 2018). Sodium, nitrogen (N) and phosphorus (P) addition results in a significant disturbance to native soil microorganisms (Jia et al. 2015; Zhu et al. 2016; Martiny et al. 2017), which then alter the litter quality and influence the variation of soil microbial communities. However, little is known about how the litter's original microbes regulate soil microbes after they are incorporated into soil.

An endophyte is an organism that colonizes the internal tissue of a plant at some stage during their life cycle without causing disease (Wilson 1995). Endophytes could modify the microenvironment for decomposition by stimulating or reducing soil microbial activity (Omacini et al. 2004). Although ubiquitous within plant tissues, endophytes can vary spatially and temporally within host plants and represent an important bridge between host plant characteristics that influence decomposition and the decomposer community (Younginger and Ballhorn 2017; Wolfe and Ballhorn 2020). During the coevolutionary process with their hosts, endophytes have developed many varieties of extracellular enzymes to maintain a stable symbiosis, and it has been proven that these extracellular enzymes participate in the degradation of plant litter (Wang et al. 2011; Baldrian et al. 2011).

First, plant endophytes act as “pioneer” decomposers because of their persistence in live, senescent or dead inner plant tissues (Yuan and Chen 2014; Snajdr et al. 2011), shortening the inoculation time during the initial stage of decomposition. The presence of endophytic fungal in litter has been shown to accelerate the inoculation of fungal decomposers under laboratory conditions (Osono 2007; Baldrian et al. 2011). However, the involvement of endophytic bacteria (EB) in the litter decomposition process has received far less attention than that of fungi. Depending on the dominance, bacteria and fungi can have significant impacts on the rate of litter decomposition. Wilkinson et al. (2000) found that litter bacterial biomass was higher than fungal biomass at an early stage than later stages of spruce litter decomposition. Soil bacteria can contribute to the breakdown of not only simple compounds such as sugars and amino acids, but also complex phenolic compounds including lignin (Větrovský et al. 2014; Brown and Chang 2014). Bacterial richness is positively correlated with hydrolytic enzymes for C, N and P acquisition during the decomposition process (Purahong et al. 2016), and are major natural agents responsible for nutrient element fixation in ecosystem processes (Reed et al. 2011). In addition, > 40% of culturable bacteria can mineralize organic P by releasing numerous phosphatases into the surrounding soil (Jorquera et al. 2008).

Furthermore, bacteria are important mediators of the P cycle because some bacteria can solubilize mineral P and mineralize organic P while others may immobilize it in their biomass (Lladó et al. 2017). Various phosphorous-soluble EB have been reported (Nath et al. 2012; Valetti et al. 2018; Ye et al. 2020). As components of key molecules such as nucleic acids, phospholipids, and amino acids, P and N are two

of the main limiting nutrients for microbial growth and activity and are also essential nutrients for plant growth and performance (Xiao et al. 2010; Fang et al. 2017). The ultimate source of N is derived from the atmosphere and is theoretically unlimited, while P is supplied mostly by weathering of minerals leading to P is the limiting nutrient in soils (Lladó et al. 2017). Enzymatic hydrolysis of the labile organic P pool by phosphatase and phytase exudation is one of the essential mechanisms of P acquisition (Fatemi et al. 2016; Giles et al. 2018; Wu et al. 2019). Phosphatase enzymes mineralize phosphrus from organic phosphate esters during organic matter decay and play an important role in the mobilization of organic forms of P (Madejón et al. 2001). Phosphatase enzymes are produced by both plants (Zhang et al. 2018; Wei et al. 2019) and microorganisms (i.e., fungi and bacteria) in soil. Unfortunately, the involvement of bacteria, especially endophytic bacteria in P cycling has received far less attention than bacterial involvement in the N and C cycling.

Our objectives of this study are to evaluate effect of EB exited in litter on soil ecosystem function. We hypothesize that the presence of EB in host litter may still play an ecological role to regulate the community structure of soil fungal decomposers, leading to enhance the decomposition of litter. Bacteria are important mediators of the P cycle because some can solubilize mineral P and mineralize organic P while others may immobilize it in their biomass (Lladó et al. 2017). Therefore, our second hypothesis is that EB entering the soil from host litter can regulate the P cycling of organic matter.

To test the hypothesis, we used the model species *Arabidopsis thaliana* to obtain litter inoculated with and without EB. *A thaliana* has been used to determine the phenotypic and genetic drivers of litter decomposability (Bergkemper et al. 2016; Kazakou et al. 2019). Various rhizosphere and endophytic bacteria are found to be associated with different developmental stages and genotypes that vary in plant growth and flowering phenology (Lundberg et al. 2012; Lu et al. 2018). An EB strain *Bacillus cereus* BCM2 was originally isolated from healthy tissue of a *Fragaria ananassa* plant. This EB strain can be steadily colonized in the rhizosphere and roots of *A. thaliana* throughout its life (Fig. S1). Besides, the EB also promoted the growth of *A. thaliana*, significantly increasing the fresh and dry weight (Fig. S2) after 2 weeks inoculation. The decomposition process of litters with and without BCM2 were studied using litter bags.

2. Materials And Methods

2.1 Soil collection

Soil was collected from an agriculture experimental field with an annual mean temperature of 15.4℃ and annual precipitation of 1106 mm (Liu et al. 2019) at Nanjing, Jiangsu Province, China (32.06°N, 118.54°E). Five 0.5×0.5 m sample plots were randomly selected, and the topsoil (0-20 cm) was collected and mixed. All the soil was sieved with 3 mm fine-structure mesh to remove plant materials and stones. Total C and N concentration of litter were determined using an elemental analyzer (Elemental Vario Micro, Germany) (Jia et al. 2015; Tian et al. 2018). Organic matter content was measured after igniting the dry samples for 5 h at 550°C (Täumer et al. 2005). Soil pH was measured using a glass electrode at a ratio of

1:2.5 (soil:water) after shaking for approximately 30 min (Dick et al. 2000). The original physical and chemical properties of soil and microbial populations are shown in Table S1.

2.2 Preparation of A. thaliana litter inoculated with and without endophytic bacteria

The EB strain was used for preprocessing the seedling of *A. thaliana* as follows: the EB was labeled with the streptomycin sulfate resistance gene (which make the EB strain can grow on plate containing 300 ug/ml streptomycin), incubated in 100 ml beef extract peptone medium (0.3% beef extract, 1% peptone and 0.5% NaCl) in a 250 ml bottle at 28°C with 160 rpm for 48 h. The cells were collected by centrifuging the medium at 5000 rpm for 3 min, then rinsed three times with sterile water. The 10 ml EB suspension in sterile water (OD_{600} = 0.536, the concentration of bacteria was 3.42×10^8 cfu/ml) was inoculated to rhizosphere of three-week old *A. thaliana* rhizosphere. *A. thaliana* inoculated with sterile water served as a control. *A. thaliana* litters (whole dead plant except seeds) with EB and without EB were collected in the end of the growth period (60 d) and were dried at 40°C to a constant weight for further uses.

2.3 Litterbag incubation experiment

A microcosm experiment was conducted at Nanjing University from November 2018 to May 2019 to examine the regulating effect of EB (*B. cereus* BCM2) on *A. thaliana* decomposition. The collected litter samples were placed in 15 cm diameter × 14 cm height plastic pots with 600 g experimental field soil. Nylon litterbags (0.3-mm mesh size, 5 × 10 cm filled with 1 g of litter of *A. thaliana*) were used for the incubation experiment to prevent litter being chewed by soil fauna (Tian et al. 2018). Litterbags were placed at the depth of 5 cm beneath the soil surface to mimic standard field decomposition condition. A treatment (T) of 60 ml BCM2 suspension with OD_{600} = 0.060 (the cell concentration was about 5.63×10^6 cfu/ml) was added to each treatment pot, to ensure a concentration (5.63×10^5 cfu/g) in the soil, which the concentration was a mean value obtained in the rhizosphere of *A. thaliana* during the growth for litter preparation (Fig. S1). The control (C) pots were inoculated with 60 ml sterile water. Soil moisture was maintained at 30-40% (v/v) and detected with a moisture meter (Ke shunda technology cot. LTD, Shenzhen, China). The culture condition was 20 ± 2°C. In total, 36 litterbags were prepared and deployed onto the treatment pots. Three litterbags were harvested each month during incubation time. And about 20 g soil samples beneath the litterbags were collected and divided into two parts, one stored in a sealed plastic bag at -20°C for high throughput sequencing and the another for laboratory analyses.

2.4 Determination of litter mass loss, C and N loss

Litter samples were rinsed gently on a 0.5 mm sieve, and oven-dried to a constant weight (40°C for 48 h) to determine mass loss. Total C and N concentrations of dried litter and soil samples were determined using an elemental analyzer, calculated as $[(M_i \times CN_i) - (M_f \times CN_f)] / (M_i \times CN_i) \times 100$ (Osono and Takeda 2002), where M_i and M_f are the initial and final incubated litter dry mass, respectively, and CN_i and CN_p are the initial and final C or N concentration (% of dry mass). The C and N addition in soil was calculated as $(S_f - S_i)/S_i \times 100$, where S_f and S_i are the final and initial C or N concentration in soil. The positive litter

C and N loss indicates net C and N release, the increase of C and N in soil indicates C and N sequestration. Total P in litter was determined by the molybdenum-antimony colorimetric method (Allen 1989; Hu et al. 2017). P content in plants was calculated as follows:

$$P\ (\%) = \frac{\rho \times V \times ts \times 10^{4}}{m}$$

Where p is the concentration of P found in the standard curve ($mg \cdot l^{-1}$), v is volume of chromogenic liquid, ts is digested liquid constant volume was absorbed in the experiment, 10^{-4} converts the mg/l to a percentage, and m is the mass of plants.

2.5 Microbial biomass and enzymatic activity measurements

Soil microbial biomass was measured using the substrate-induced respiration (SIR) method (Bailey et al. 2002; Jia et al. 2015). Briefly, three samples of 1 g soil were respective placed in glasses vial (100 ml) and incubated with glucose (10 mg glucose g^{-1} soil dry weight) at 25°C. CO_2 production was detected two times through an infrared gas analyzer (DISLab LW-B802, Shanghai Digital Experiment System R&D Center) after 1 h of glucose addition. The population of EB in the soil surrounding *A. thaliana* litter incubation sites was assessed with 1 g soil dilution coated on plate containing 300 ug /ml streptomycin at each harvest time. We assayed the potential activities of nine extracellular enzymes involved in litter C and nutrient cycling (Talbot et al. 2012; Tian et al. 2018), including cellobiohydrolase (CBH1 71123), β-1,4-glucosidase (BG), β-1,4-xylosidase (BX), nitrate reductase (NR), urease (URE), acid phosphatase (ACP), alkaline phosphatase (ALP), peroxidase (POD) and phenol oxidase (Phox). Detailed descriptions of the assay methods were referred to Tian et al. (2018), the detail description is available in the supplement file.

2.6 DNA extraction and Illumina MiSeq sequencing of the 16S rRNA and ITS gene

Soil genomic DNA was extracted by the Mobio Po werSoil DNA isolation kit (MoBio Laboratories, Inc., USA). The DNAs extracted from 24 soil samples which from EB and control treatments after decomposition of 1, 2, 4 and 6 months, have been sequenced by Huada Genomics Institute (BGI, Shenzhen, China). Primer set 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') was used to amplify the bacterial V4 region of 16S rRNA genes (Caporaso et al. 2011, Apprill et al. 2015), and primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') was used to amplify the Internal Transcribed Spacer 1 (ITS1) of fungi (Xiong et al., 2017), which were selected for many large-scale microbiomes sequencing. PCR was performed following previously published amplication conditions (Xiong et al., 2017). PCR products were purified by Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). After assessing the library quality and quantity (Agilent Bioanalyzer 2100 system, Agilent Tech Inc., Santa Clara, USA). The 16S rRNA gene and ITS gene fragments were sequenced using the Illumina Hiseq platform.

2.7 Bioinformatic analyses

The raw sequence data were assigned to each sample according to the unique barcodes, and then removed the barcodes and the primer sequences. The obtained raw of 16S rRNA and ITS sequence data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al. 2010). Briefly, an over 25-bp window size was used to trim the unqualified sequences using BTRIM (Kong 2011), removing joint contamination reads (with at least a 15-bp overlap and < 0.1 mismatches). The obtained sequences were normalized to the minimum number of reads across all samples for the downstream analysis. Bacterial and fungal sequences were then independently clustered into operational taxonomic units (OTUs) at a 97% identity threshold using UPARSE (v7 .0.1090) (Edgar 2013). The chimera produced by PCR amplification was removed using UCHIME (v4.2.40). Taxonomy of bacteria was assigned to each sequence through BLASTing of the RDP (http://rdp.cme.msu.edu/) classifer (v2.2) (Cole et al. 2009) with the confidence threshold is set to 0.8, and Greengene (http://www.greengene.com), UNITE (https://github.com/downloads/qiime/its-reference-otus/its_12_11_otus.tar.gz) were database for fungi (Nilsson et al., 2018).

The α-diversity of soil bacteria and fungi, including the number of observed Chao1 richness and Shannon's diversity index, was calculated (Dini-Andreote et al. 2014) using alpha_diversity.py script of QIIME (http://qiime.org/scripts/alpha_diversity.html). The bacterial and fungal DNA sequences of 24 soil samples have been deposited in the SRA of the NCBI database under the accession of PRJNA624026.

2.8 Activity of phosphate solubilization and phosphatase production of EB

The original EB solubilizing activity of organophosphorus and inorganic phosphorus was assessed after litter decomposition using the dissolved phosphorus cycle method with modified National Botanical Research Institute's phosphate growth medium (NBRIY) (Nautiyal 1999; Jorquera et al. 2008) containing glucose, 10 g/l; $Ca_3(PO_4)_2$, 5 g/l; $(NH_4)_2SO_4$, 0.3 g/l; NaCl, 0.2 g/l; $MgSO_4 \cdot 7H_2O$, 0.3 g/l; KCl, 0.2 g/l; $MnSO_4 \cdot H_2O$, 0.002 g/l; $FeSO_4 \cdot 7H_2O$, 0.002 g/l and agar, 20 g/l. To detect the ability of organophosphorus dissolution, the EB strain was incubated using basic Pikovskya's (PVK) agar medium with lecithin: glucose, 10 g/l; $(NH_4)_2SO_4$, 0.3 g/l; lecithin 1 g/l; NaCl, 0.2 g/l; $MgSO_4 \cdot 7H_2O$, 0.3 g/l; KCl, 0.2 g; $MnSO_4 \cdot H_2O$, 0.002 g/l; $FeSO_4 \cdot 7H_2O$, 0.002 g/l; yeast extract, 0.4 g/l and agar, 15 g/l). Petri plates were incubated at 28°C for 120 h, and the phosphorus hydrolysis circle were recorded.

The alkaline and acid phosphatase production capacity of the EB strain was detected as follows: bacteria were incubated in beef extract peptone medium at 28°C for 48 h. Then, aliquots of each sample were added to 0.48 ml of 0.1 M universal buffer with pH 6.5 or pH 11, respectively, for acid and alkaline phosphatase activity, and 0.12 ml p-nitrophenyl phosphate (pNPP) 0.05 M solution, followed by 1 h incubation at 37°C. A control treatment containing only liquid medium was included in each experiment with pNPP added after incubation. The yellow color was detected at 410 nm (Tabatai and Bremmer 1969; Oliveira et al. 2009). In order to detect phosphatase activity during the litter decomposition, 0.2 g of *A. thaliana* litter was added into 100 ml de-nutrition beef extract peptone medium (ten times dilution) incubated at 28°C for 48 h. The control added 0.2 g *A. thaliana* litter not infected with BCM2. Then, alkaline and acid phosphatase production activity was detected as above.

2.9 Available phosphorus in soil during litter decomposition and total P in plants

Available phosphorus (A-P) in harvest soil was determined using the molybdate colorimetric method after ascorbic acid reduction (Allen 1989). A 2 g sample of air-dried soil passed through a 20-mesh sieve (bore diameter 850 μm) was used to detect available phosphorus detection. Then, 50 ml of 0.5 mol/l $NaHCO_3$ solution was added to each sample and agitated, shaken for 30 min, and filtered with phosphorus-free filter paper. We pipetted 10 ml of filtrate, added 35 ml of distilled water to dilute the sample, and then added 5 ml of molybdenum antimony anti-chromogenic agent. The solution was mixed well and allowed to stand for 30 min, and the blue color was measured at 880 nm. Total P was determined by the molybdenum-antimony colorimetric method after the samples were digested with H_2SO_4 and H_2O_2 (Allen 1989; Hu et al. 2017). The 5 ml digestion was diluted to 50 ml, first adding 2 drops of 2, 4-dinitrophenol indicator, and then 6 mol NaOH was used to neutralize the digested liquid to just yellow. We then added 1 drop of 2 mol H_2SO_4 to fade to yellow, and added 5 ml of molybdenum-antimony anti-chromogenic agent. The solution was mixed well and allowed to stand for 30 min, the blue color was measured at 880 nm. The content of available phosphorus in soil was determined using the following equation:

$$A\text{-}P\ (mg \cdot kg^{-1}) = \frac{\rho \times V \times ts}{m \times k \times 10^3} \times 1000$$

Where is the concentration of P found in the standard curve (μg·ml^{-1}), *v* is the volume of constant volume during color rendering, *ts* is the fraction multiple (total volume of extraction extract absorbed in the experiment), *m* is the mass of air-dried soil, *k* is conversion coefficient from air-dried soil to drying soil, 10^3 is to convert the μg to mg, 1000 is to convert the concentration to P per kilogram.

2.10. 'After-life effect' on wheat growth by litter with EB decomposition

To study litter decomposition feedbacks to subsequent plant growth in the ecosystem, 5 wheat (*Triticum aestivum* L.) seedlings were planted into each soil after litter incubation. Soil moisture was maintained at 30-40%, and temperature at 20 ± 2°C. Fresh and dry weight of wheat were measured after each month. The total P content of wheat after one month as also determined by the molybdenum-antimony colorimetric method (Allen 1989; Hu et al. 2017).

2.11 Data analysis

The constant of potential mass loss rate over time was determined using an exponential equation (Olson 1963):

$$k = \frac{-ln\ (x_t/x_0)}{t}$$

Where x_o is the original mass of litter, x_t is the amount of litter remaining after time t (month), and *k* is the litter decomposition coefficient (month^{-1}).

We used independent sample t tests to assess differences between the treatment and the control group in this study. The correlation of EB richness in soil and SIR was based on the Pearson's product-moment correlation. The effect of A-P on growth parameters of wheat was evaluated using semi-parametric permutational multivariate ANOVA (PERMANOVA,) (Anderson 2001) using the 'vegan' package in R. The variation in the bacterial and fungal community caused by six-month decomposition and EB treatment was investigated using Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities using QIIME (Caporaso et al. 2010). To detect the effect of EB on the soil microbial community, SIR, soil enzyme activity, litter decomposition, A-P in soil and subsequent plant P content, and minimized the confounding interactions among causal factors, a structural equation model (SEM) was implemented to further reveal the possible pathways and interactions between the factors through a priori modeling (Fig. S3), using R package "lavaan" (Rosseel 2012). Each pathway in the model was evaluated for significant contributions to the model. Indices of model fit were the chi-square test (a lower chi-square indicates a better model), with P traditionally > 0.05, the root mean square error of approximation (RMSEA; the model has a good fit when RMSEA <0.05) and the 90% confidence intervals (Oberski et al. 2014).

3. Results

3.1 Litter decomposition

During the 6 months incubation, *A. thaliana* litter decomposed quickly, with nearly 92 % and 87 % of litter mass loss for litter with EB and the control, respectively. Litter with EB (k = 0.42 month^{-1}) decomposed more quickly than the control (k = 0.34 month^{-1}) (Fig, 1B). However, there was no significant difference between these rates throughout the decomposition period. Upon reviewing the decomposition process, we found that the cumulative mass remaining of litter with the EB treatment was significantly lower than in the control treatment in the second to fourth month (Fig. 1A, P = 0.03, P = 0.01 and P = 0.007, respectively), but there was no significant effect of the treatment at the beginning and anaphases of degradation.

3.2 The abundance of EB during litter decomposition and its interaction with SIR

During the six-month incubation, the average SIR of the EB treatment was higher than the control in the middle stage of decomposition, especially at the third month (P = 0.0091), when the EB treatment significantly enhanced the SIR of soil (Fig. 2A). We also surveyed the population of EB around litter over the six months (Fig. 2B). In general, the number of bacteria was stable after the first stage of decomposition, with a level of $\geq 10^5$ CFU/g in the soil. However, we found a strong negative correlation between EB richness and SIR (r = −0.6009, P = 0.0084), indicating that a large number of bacteria corresponded to a low soil respiration value. Due to the fluctuations of respiration in soil driven by indigenous soil microorganisms and a negative contribution of litter decomposition by EB on soil SIR, suggesting that EB may regulate the native microbes.

3.3 Enzyme activities

In general, most extracellular enzyme activities involved in C, N and polyphenol cycling were not up-regulated by the EB treatment compared to the control in the soil (Fig. S5). The soil enzyme activity of ALP was significantly higher in the EB treatment than in the control in the first and second stages of decomposition ($P < 0.05$, Fig. S5). The first two components (PC1 and PC2) of the PCA explained 46.5% and 22.2% of the variance respectively (Fig. 3). Phenol-related enzyme (PhOx and Pero) activities were strongly correlated with litter decomposition rates in the early stage. P-related enzyme (ALP and ACP) activities were correlated with the early and middle decomposition stages. The contribution of C-related (CBHI, BG and BX) and N-related (URE and NR) enzymes on litter decomposition is concentrated in the middle and late stages.

3.4 Soil microbial community composition, α- and β-diversity

3.4.1 Soil bacterial and fungal community composition

A total of 45684-44348 bacteria OTUs and 3426-4035 OTUs per sample were obtained in the EB treatment and the control, respectively (Table S2). The number of OTUs in soil significantly increased in the litter with EB decomposition compared with the control ($P = 0.0202$, n = 3). A total of 7123, 7137 fungi OTUs and 479-726 OTUs per sample were obtained in the treatment and control, respectively, through OTU clustering analysis (Table S2). EB released from litter decomposition had no significant effect on the fungi OTUs ($P = 0.9649$, n= 3).

3.4.2 Diversity of soil bacteria and fungi

The EB treatment significantly promoted the richness of bacteria during six months of decomposition (Fig. 4A). The richness of bacteria at middle and late decomposition stages was significantly higher than that in the early stage (Fig. 4A). EB significantly increased the richness and diversity of soil bacteria at the middle decomposition stage (Fig. 4A, B; Chao 1, $P = 0.0008$; Shannon, $P = 0.0459$). EB treatment did not change the richness and diversity of fungi compared with the control during six-month incubation (Fig. 4C, D, $P > 0.05$). The richness of fungi was higher in the middle stage of degradation. Shannon's index showed significant differences in the EB treatment during six-month decomposition and increased in the middle and late stages (Fig. D). This result indicates that EB released from litter into soil regulated the native microbial community and mainly concentrated in the middle stage of decomposition.

3.4.3 Community structure of soil bacteria and fungi

Community comparisons by PCoA of each group showed that the bacterial and fungal communities were exhibited separate trends between the treatment and control according to their different treatments and decomposition time (Fig. 5), suggesting that EB released from decomposing litter caused distinct community assembly of native microbes. EB treatment resulted in significant differences in the bacterial and fungal communities compared to the control in the fourth month of decomposition. Different bacterial groups in the treatment (T6, six months after decomposition) and control (C6, six months after decomposition) clustered together at the late stage of decomposition (Fig. 5A). However, the community

differences of fungi remained large in the later stage (Fig. 5B). This result suggests that the bacteria in the treatment and control in the late stage of degradation tended to recover similar community structure after succession.

3.5 Species composition and relative abundance at different decomposition stages in soil

The histogram shows the soil bacterial community at the family level between the EB treatment and control during litter decomposition (Fig. 6A). Nitrososphaeraceae (an ammonia-oxidizing archaea, AOA) was the most abundant bacterial family across the whole decomposition process, and displayed an increasing successional trend with degradation time. In addition, three types of decomposer bacteria (Comamonadaceae, Oxalobacteraceae and Rhodospirllaceae) were more abundant in the middle decomposition stage in the EB treatment. Bacillaceae relative abundance significantly increased at the early stage of decomposition (Fig. 6A, group T1, $P = 0.0422$). There was a similar trend at the order level. For instance, Nitrososphaerales was the most abundant order during the six-month incubation (Fig. S6A), and the EB treatment did not change its overall abundance compared to the control (Mean abundance: T = 11.35%, C = 12.27%, $P = 0.3033$). The relative abundance at the order level showed that Bacillales in group T1 (treatment litter at one month of decomposition) was more abundant than other orders (Fig. S5A). This noteworthy phenomenon was consistent with the observations of EB richness in the soil, which indicated that EB richness peaked in the first month (Fig. 2B). Pearson correlation showed that there was a stronger but non-significant correlation between EB richness and Bacillaceae ($r = 0.928$, $P = 0.072$), suggesting that native Bacillaceae in the soil contribute to the relative abundance of Bacillaceae overall.

The result of the histogram of species abundance shows that EB addition affected fungal community composition. (Fig. 6B). The most abundant fungal family at the early decomposition stage was Mortierellaceae, which had a relative abundance of 36.7% and 37.6% in the EB treatment and control, respectively. In the EB treatment, its dominant status was replaced by the Nectriaceae (21.78%) in the later successional stage, but not in the control, and the mean relative abundance of Mortierellaceae in the treatment (19.06%) was significantly lower than in the control (27.48%) over the six-month incubation ($P = 0.0383$). In addition, the relative abundance at the order level displayed a similar trend, which showed that the most abundant fungi, Mortierellales, in the treatment was gradually replaced by Hypocreales (Fig. S6B) at the later stage of decomposition. The mean relative abundance of Ascobolaceae (saprotrophic fungi) was significantly higher than that of other orders (ANOVA, $P = 0.0006$) in treatment T1 group (7.6%, Fig. 6B). In Fig. S5B, the relative abundance at the order level of Pezizales (Ascobolaceae affiliated to this family) was the highest in T1. In addition, the relative abundance of Pleosporaceae, Orbiliaceae, Pyronemataceae, Nectriaceae, and Pseudeurotiaceae belong to Ascomycota were up-regulated in the EB treatment in T2 an T4 (Fig. 6B), suggesting a disturbance of litter with EB fall out for native fungi in middle stage of decomposition.

3.6 Activity of EB in relation with phosphorus and phosphatase production

Phosphorus availability was elevated by EB treatment through both dissolving inorganic P and degrading organic P (Fig. 7). EB demonstrated a strong ability to degrade organic phosphorus. The hydrolysis circle reached 5.28 mm after 96 hours of cultivation (Fig. 7A). The degradation ability of inorganic phosphorus was relatively weak, and the hydrolysis circle reached 1 mm after 120 hours of cultivation (Fig. 7B). The EB strain in this study produced more alkaline phosphatase than acid phosphatase (P <0.0001, Fig. 7C). The results of co-cultivation with litter showed that the enzyme activities of ALP and ACP increased by about 45 times, and the enzyme activity of ALP was significantly higher than that of ACP (P <0.0001, Fig. 7C). Because litter contains organophosphorus compounds such as nucleic acids and phospholipids, this suggests that EB plays an important role in the litter degradation process and P mineralization.

3.7 Available phosphorus in soil and total P in litter over six months of decomposition

In general, available P content was significantly higher in the EB treatment than in the control in all but the first month of the six months decomposition period (Fig. 8A, P < 0.01). In addition, A-P content in the treatment at the later stage was significantly higher than in the former stage of the litter decomposition process (P = 0.0092). There was no significant difference in A-P content within the control group (P = 0.117), which suggests that the EB treatment promoted the accumulation of A-P in soil. After six-month decomposition, P content of *A. thaliana* litter in the EB group and control group decreased by 97.71% and 77.83%, respectively (Fig. 8B). The loss of P in litter with EB significantly increased compared with control (P = 0.0011).

3.8 Litter with EB 'After-life effect' on wheat plant growth

Wheat growth in soil with the EB treatment was higher than in the control (Table 1). Litter with EB decomposition significantly increased the fresh and the dry weight of wheat at the later stage of decomposition (Table 1). In addition, litter with EB increased the accumulation of total P in wheat biomass (Fig. 8B). However, there was no significant difference between the treatment and the control (P = 0.4229). The PERMANOVA correlation analysis showed that there was a significant correlation between wheat growth parameters and soluble P (Pr (> F) = 0.001), suggesting that EB increased the 'after-life effect' of host litter on subsequent plant growth.

3.9 Interactions of EB, soil microbes, litter decomposition, A-P and wheat growth

According to SEM, EB had direct impact on bacterial richness, soil enzyme and available P in soil, which explained 55% (P < 0.01), 49% (P < 0.001) and 45% (P > 0.05) of the variation, respectively (Fig. 9). Bacterial community in soil directly affected available P and SIR, explaining 25% (P > 0.05) and 43% (P > 0.05) of variation, respectively. In addition, bacterial community had a negative effect on litter decomposition (53%, P < 0.01) and soil enzyme activity (69%, P < 0.001). However, soil enzyme activity and litter decomposition were mainly affected by fungal richness in soil, which explained 24% (P < 0.05) and 0.9% (P > 0.05) of respective variation. Litter decomposition was directly influenced by EB (30%, P < 0.05) or indirectly affected by regulation of fungal community structure and soil enzymes. Subsequent

plant growth after litter decomposition was subject to impacts of EB (33% P <0.01), bacterial richness (48%, P <0.01) and A-P (13%, P > 0.05).

4. Discussion

4.1 EB mediated A. thaliana litter decomposition

In this study, we recorded nearly 92% and 87% mass loss in the EB treatment and control, respectively, after six months of decomposition, indicating that *A. thaliana* reached the late stage of decomposition after six months in the laboratory. The process of decomposition can be clearly divided into three decomposition stages: the early, middle, and last stage. The decomposition rate of *A. thaliana* litter was much higher than that of other species such as angiosperms *Quercus variabilis* (Jia et al. 2015; Tian et al. 2018) and *Schima superba* (Pietsch et al. 2019), gymnosperm *Pinus massoniana* (Jia et al. 2015; Tian et al. 2018; Pietsch et al. 2019) in forests, and hydrophyte *Typha angustifolia* found in an aquatic ecosystem (Kong et al. 2018). *A. thaliana* litter mass loss and decomposition rate (*k* value) was higher in the EB treatment than in the control after six months of incubation (Fig. 1). SEM results also indicated that EB directly affected the decomposition rate (Fig. 9), which further supports the hypothesis that EB promotes the decomposition of host plant litter. Litter quality including N concentration was lower and litter C:N was higher in the treatment compared to the control (Fig. S4) after decomposition. However, there was no significant difference in soil N between the treatment and control. This result implies that EB significantly promoted *A. thaliana* litter decomposition and N release. suggesting that EB mediated *A. thaliana* N loss, but did not translate into a similarly strong influence on soil N immobilization.

Frey-Klett et al. (2011) posited that bacteria probably facilitate fungal decomposers by providing electrons or essential micronutrients and are thus indispensable for litter degradation, while some other studies postulate that fungi regulate the niche of bacteria, which simply colonize the soil-litter interface to take advantage of readily available substances degraded by fungal extracellular enzyme in litter (Boer et al. 2005; Romaní et al. 2006). Recent studies suggest that diverse bacteria and fungi co-occur and may interact during the course of litter decomposition (Purahong et al. 2015; Urbanová et al. 2015). The strain used in this study is an infective endophytic bacteria of model organisms, but our results indicate that it is also a bacterium that promotes the decomposition of model plant *A. thaliana*, with two major functions of endogenous and decomposition promotion (Fig. S1, Fig. 1 and Fig. S4). Our work supports the hypothesis that EB mediate native microbial community composition (Fig. 5, Fig. 6 and Fig. 9) or directly participate in litter degradation (Fig. 1, Fig. 9) of *A. thaliana*.

4.2 Litter with EB changed soil enzyme activity in the early stage of decomposition

Enzymatic activity was demonstrated to be the main driver of litter-decomposer interactions that modify the trajectory of litter mineralization (Fanin et al. 2016). PhOx and Pero were highly correlated with the litter degradation rate in the first month (Fig. 3). Polyphenols are commonly viewed as a group of secondary plant metabolites that typically inhibit decomposition (Hättenschwiler et al. 2005). Plant cell walls consist of a complex composite of the various hemicelluloses, polysaccharides cellulose, pectic

polysaccharides, and the polyphenol lignin, and *A. thaliana* is rich in polyphenols (Jamalnasir et al. 2013; Ghura et al. 2016). A short contribution of PhOx and Pero to litter decomposition at the early stage possibly leads to the promotion of subsequent degradation. As Duan et al. (2019) and Steinweg et al. (2008) reported, increases in phosphatase were likely due to increased microbial activity. Most soil extracellular enzyme activity involved in C and N cycling did not significantly differ between the EB treatment and the control (Fig. S4, Fig. S5). However, the activity of ALP was remarkably high in the EB treatment at the early and middle stages of decomposition (Fig. 3), which indicated that the activation of P-related enzymes by EB appeared in the early stage of degradation.

4.3 Litter with EB mediated the soil microbial community

We hypothesized that EB released in the soil from host litter can directly lead to the optimization of the soil microenvironment and regulate the structure of the soil fungi community. In our study, *A. thaliana* litter with EB incubation significantly increased the soil bacterial richness, but had low impacts on the bacterial diversity index (Fig. 4, Fig. 9). Apart from this, the richness and diversity indices of bacteria in the second to fourth months of decomposition were significantly higher than in the control (Fig. 4, Chao 1, $P = 0.0008$; Shannon $P = 0.0459$), indicating that bacteria dominate at the middle decomposition stage. Studies have examined microbial succession during leaf litter decomposition and found that soil bacteria are more responsive to newly fallen plant litter than fungi (McMahon et al. 2005; Champman et al. 2013), which was further supported by our study. Therefore, our results partly support our first hypothesis that EB can fall into the soil with host plant and have a greater impact on soil bacterial community than that fungal. In this study, litter with EB directly affected microbial community structure during litter decay (Fig. 4, Fig. 5, Fig. 6 and Fig. 9).

The resilience of microbial communities is a key factor to evaluate the stability of ecological systems. Bacterial community composition was generally more resilient than fungal composition (Martiny et al. 2017). The soil bacteria groups in the EB control clustered together at sixth months of incubation, but there was unclear clustering of the fungal community during incubation (Fig. 5). In addition, the dominant bacteria is always of Nitrososphaeraceae, both in the EB treatment and control. However, fungi included a range of taxa, from dominant to rare in relative abundance (Fig. 6), which may suggest the bacteria had greater stability and stronger recovery after interference than fungi. Although EB mainly affect the bacterial richness in the metaphase of degradation, fungal diversity changed significantly during the six-month degradation process in the EB treatment. The fungi community in EB treatment shifted from a Mortierellaceae dominated in decomposition early soils to a more Nectriaceae dominated in decomposition later soils, and the other five taxa belonging to Ascomycota (Pleosporaceae, Orbiliaceae, Pyronemataceae, Nectriaceae, Chaetomiaceae and Pseudeurotiaceae) were slightly increased in the EB treatment (Fig. 6B). As Chen et al. (2020) reported, rare taxa can have an over-proportional role in biological processes. Thus, in this study, the decomposition rate of litter with EB may be mainly driven by low abundance of ascomycetes. This mechanism and ecological function of a community shift in response to EB released from litter should be continue to be studied.

4.4 EB promoted litter P release

EB tended to reduce the N content of decaying litter (Omacini et al. 2004; Chen et al. 2013), but little is known about the role of endophytes in litter P release. P return by litterfall and litter decomposition by the rhizospheric processes of plant roots and microbes is an essential link in soil P recycling (Comerford, 2005; Barton and Northup, 2011). Enzyme hydrolysis of organic P is one mechanism of P acquisition, by which phosphatase enzymes release phosphate from organic phosphate and esters into the soil during organic matter decay (Barton and Northup, 2011; Wu et al. 2018). The EB strain used in this study has a strong ability to degrade organic P and significantly enhanced production of phosphatase after decomposition of *A. thaliana* litter (Fig. 7). We further linked phosphatase activity to A-P content in soil. Phosphatase activity increased in early and middle stages of litter decomposition, promoted the accumulation of A-P in the middle and late stages, and accelerated the release of organophosphorus in litter (Fig. 8). The main mechanism responsible for P uptake by bacteria is the solubilization and immobilisation of inorganic P, which is the most abundant source of P (Lladó et al., 2017). The ability to solubilize inorganic P has been described for different bacteria and ectomycorrhizal fungi present in the rhizosphere and soil (Richardson and Simpson, 2011). Compared with organic phosphorus, EB in this study have a less obvious ability to degrade inorganic phosphorus (Fig. 7), but their contribution to the increase of A-P in soil is not negligible and merits further study. Overall, in accordance with our second hypothesis, endophytes released to the soil with host litter can optimize the P mineralization process of *A. thaliana* litter.

4.5 'After-life effect' of decomposition of litter with EB on the ecosystem

In terrestrial ecosystems, above- and belowground plant litter input constitutes a major resource of energy and matter (Hättenschwiler et al. 2005). *A. thaliana* litter with EB changed N release and accelerated P release from litter (Fig. S4, Fig. 8 and Fig. 9). Soil bacterial and fungal diversity play dominant roles in strengthening soil feedbacks and increasing P recycling (Wu et al. 2018). In this study, soil bacteria richness increased the availability of the soil A-P. In addition, wheat growth parameters were correlated with A-P content, and high A-P in soil promoted P accumulation in wheat. Changes in microbial community structure altered subsequent soil enzyme activity and litter decomposition, which affected the subsequent growth rate of shrubs (Elgersma et al. 2012). Our results further support that litter with EB caused legacy variation directly and indirectly by regulating the structure of the bacterial and fungal community and mediating the A-P content in soil. This legacy effect has functional consequences for decomposition and can feedback to promoted the nutrient cycling and subsequent growth of wheat (Fig. 9). In sum, the EB treatment altered the 'after-life effect' of model plant *A. thaliana* litter, and promoted subsequent plant growth by affecting microbial structure and feedbacks to litter decomposition and nutrient recycling.

5. Conclusions

To the best of our knowledge, this is the first study to explore how endophytic bacteria mediate host plant litter decomposition. Our results highlight that the model plant *A. thaliana* is also a good model for studying the degradation process of litter. The EB strain used in this study regulated the degradation process of host litter into soil by priming soil enzyme activity and the microbial community. *A. thaliana* litter with EB mediated variation in the soil microbial community, and mainly increased the richness of bacteria and variation of Ascomycota in the middle stage of decomposition. Interestingly, the EB strain had a strong ability to degrade organic P, which facilitated host litter P release. Accordingly, PAL activity and A-P content in soil were significantly increased in the EB treatment. Afterwards, EB released from host litter had the 'after-life effect' to promote subsequent plant growth by affecting microbial structure and feedback to litter decomposition and nutrient recycling. The results provide some insight into the litter decomposition in relation to endophytic bacteria.

Declarations

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Authors' contributions

Haijing Hu planned and designed the research, writing the manuscript. Rumeng Ye, Lu Pang and Han jiang performed experiments, Kai Tian and Yang Gao made experiment design verification and data analysis. Yanli Ji, Junbo Yang and Pengwei Wan prepared experiment materials. Xiaoming Zou modified the manuscript. Xingjun Tian provided project support and modified the manuscript.

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Consent to participate:

All authors listed in this manuscript all agree to participate.

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Tables

Table 1 Wheat biomass growth and statistics from independent sample t-test analyzing the 'after-life effect' of *A. thaliana* litter with endophytic bacteria decomposition

Month	Fresh weight (g/?)		*P* value	Dry weight (g/?)		*P* value
	T	C		T	C	
One	0.28 ± 0.03	0.21 ± 0.02	**0.0054**	0.03 ± 0.006	0.02 ± 0.002	**0.0188**
Two	0.5 ± 0.03	0.26 ± 0.03	**<0.0001**	0.04 ± 0.003	0.03 ± 0.005	0.1513
Three	0.56 ± 0.04	0.36 ± 0.03	**0.0010**	0.05 ± 0.004	0.04 ± 0.004	0.3076
Four	0.74 ± 0.12	0.44 ± 0.02	**0.0149**	0.11 ± 0.13	0.08 ± 0.003	**0.0357**
Five	0.76 ± 0.14	0.44 ± 0.07	0.0667	0.11 ± 0.015	0.07 ± 0.009	**0.0456**
Six	0.56 ± 0.05	0.37 ± 0.06	**0.0299**	0.08 ± 0.007	0.05 ± 0.007	**0.0033**

Note: T = EB treatment soil, C = no EB treatment soil. The bold data in the table indicate significantly different ($P < 0.05$) between treatment and control with independent sample t test.

Figures

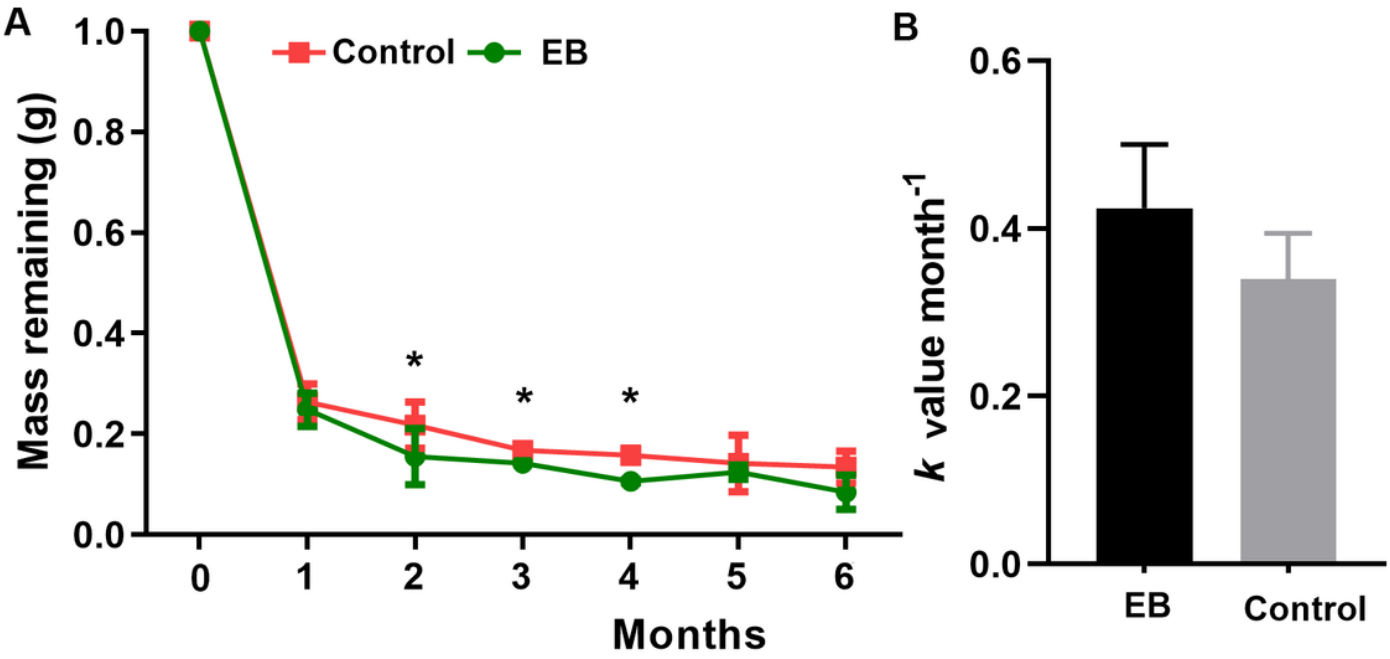

Figure 1

Decomposition of A. thaliana litter under two treatments during six-month incubation. Error bars indicate standard deviation (SD, n = 3), "*" denotes a significant difference between treatments, EB = litter with endophytic bacteria. (A) Changes in cumulative mass loss of the two treatments; (B) The decomposition rate (k month-1) of the two treatments over six months of decomposition.

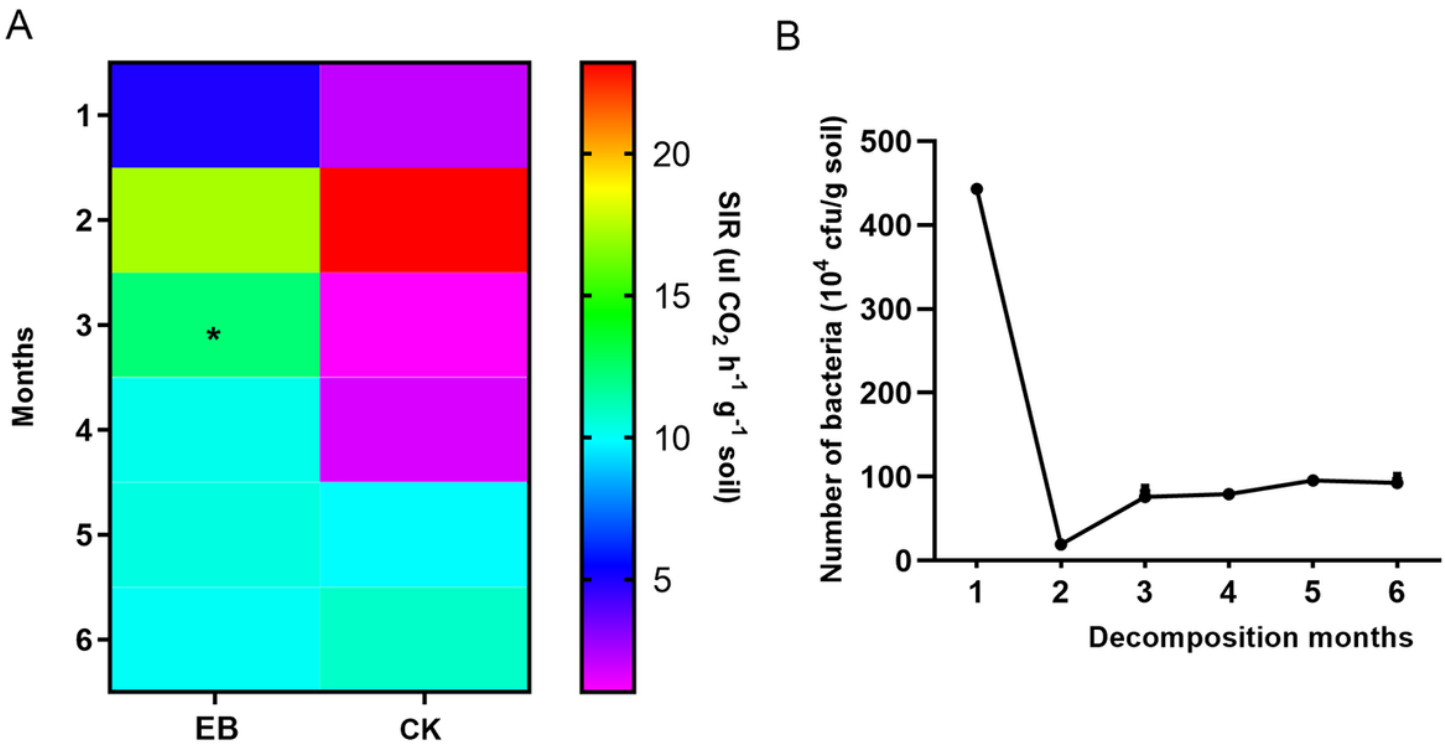

Figure 2

Soil microbial biomass in two treatments post-inoculation over six months of decomposition. Error bars indicate standard deviation (SD, n = 3), “*” denotes significant difference between treatments. (A) SIR rate of soil microbes over six-month incubation; (B) EB population in the decomposition system during six-month incubation.

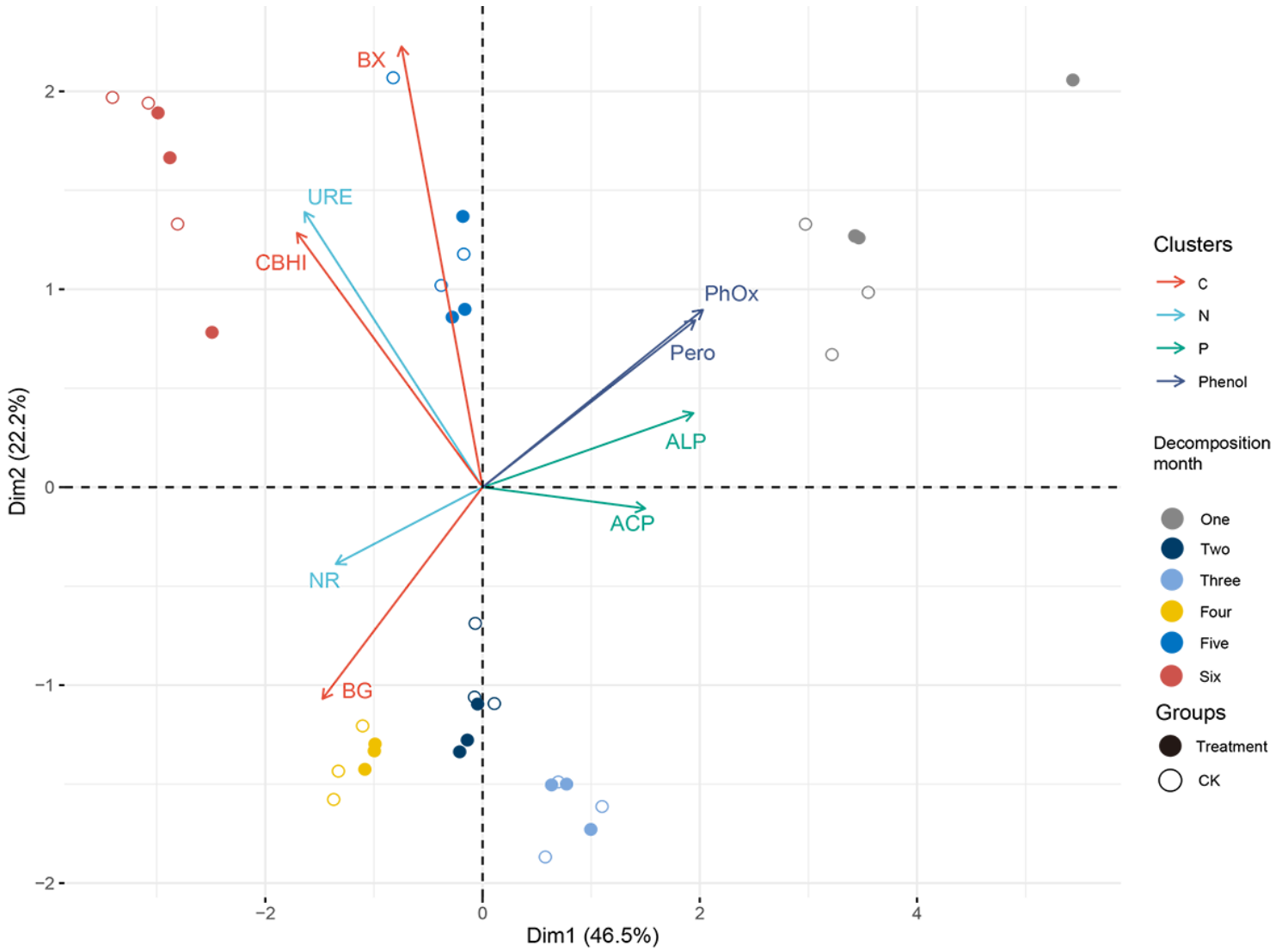

Figure 3

Principal components analysis maps based on the interactions of different functions enzyme contribute to the A. thaliana litter decomposition rate during six-month incubation. CBHI - cellobiohydrolase; BG - b-1,4-glucosidase; BX - b-1,4-xylosidase; Ure - urease; NR - nitrate reductase; ACP - acid phosphatase; ALP - alkaline phosphatase; Perox - peroxidase; PhOx - phenol oxidase.

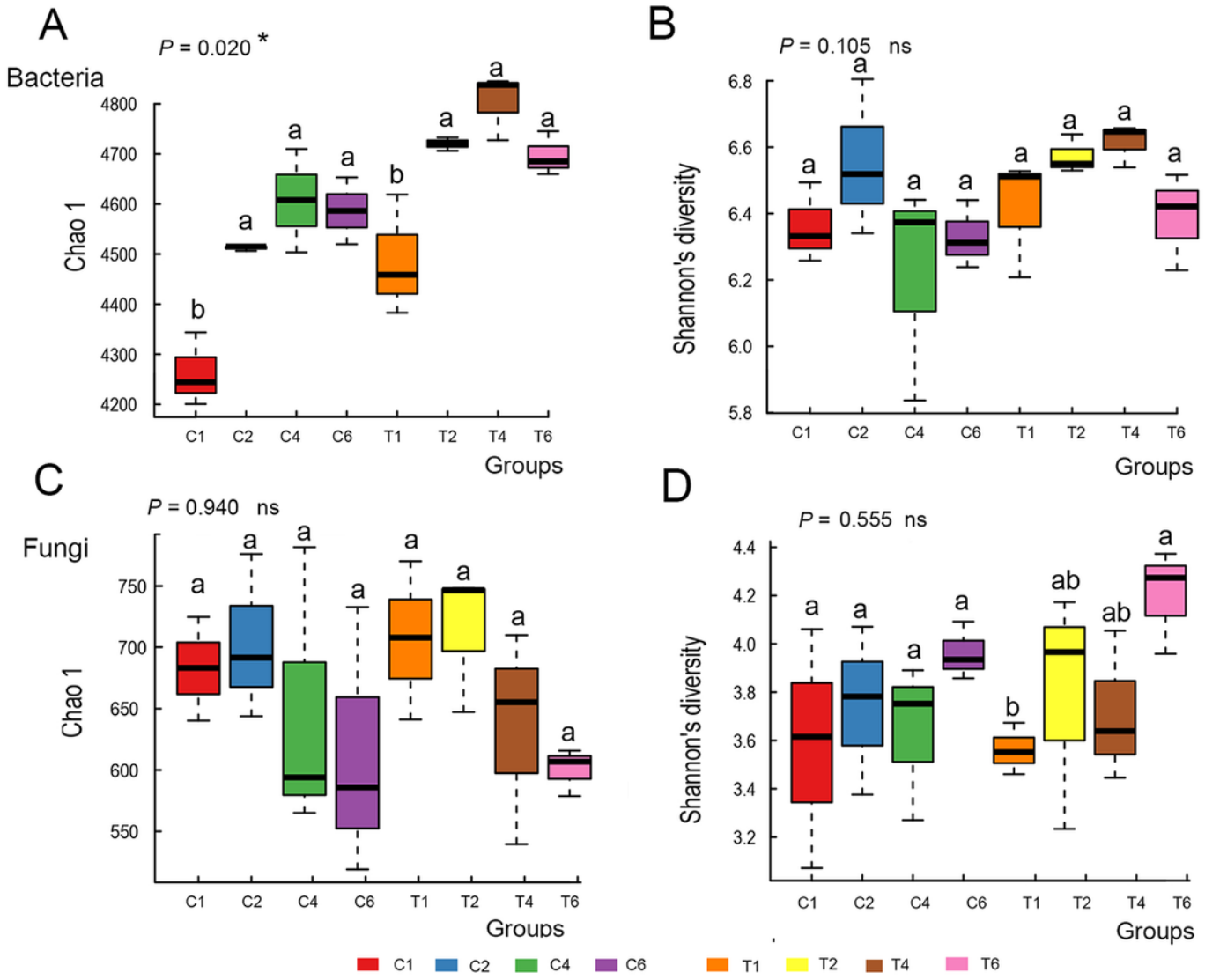

Figure 4

Bacterial and fungal α-diversity measurements in soil at different stages of decomposition (1, 2, 4 and 6 months after incubation) of A. thaliana litter. C = control, T = EB, P value is the significance level of independent sample T-test comparing the EB treatment and control, “*” denotes a significant difference, “ns” means no significant difference; different letters above box indicate significant differences (P < 0.05) determined by Turkey pairwise comparisons between treatment and control; Chao 1 represent soil microbial richness, Shannon represent soil microbial diversity; (A) Chao 1 index of bacteria; (B) Shannon’s index of bacteria; C. Chao 1 index of fungi; D. Shannon’s index of fungi.

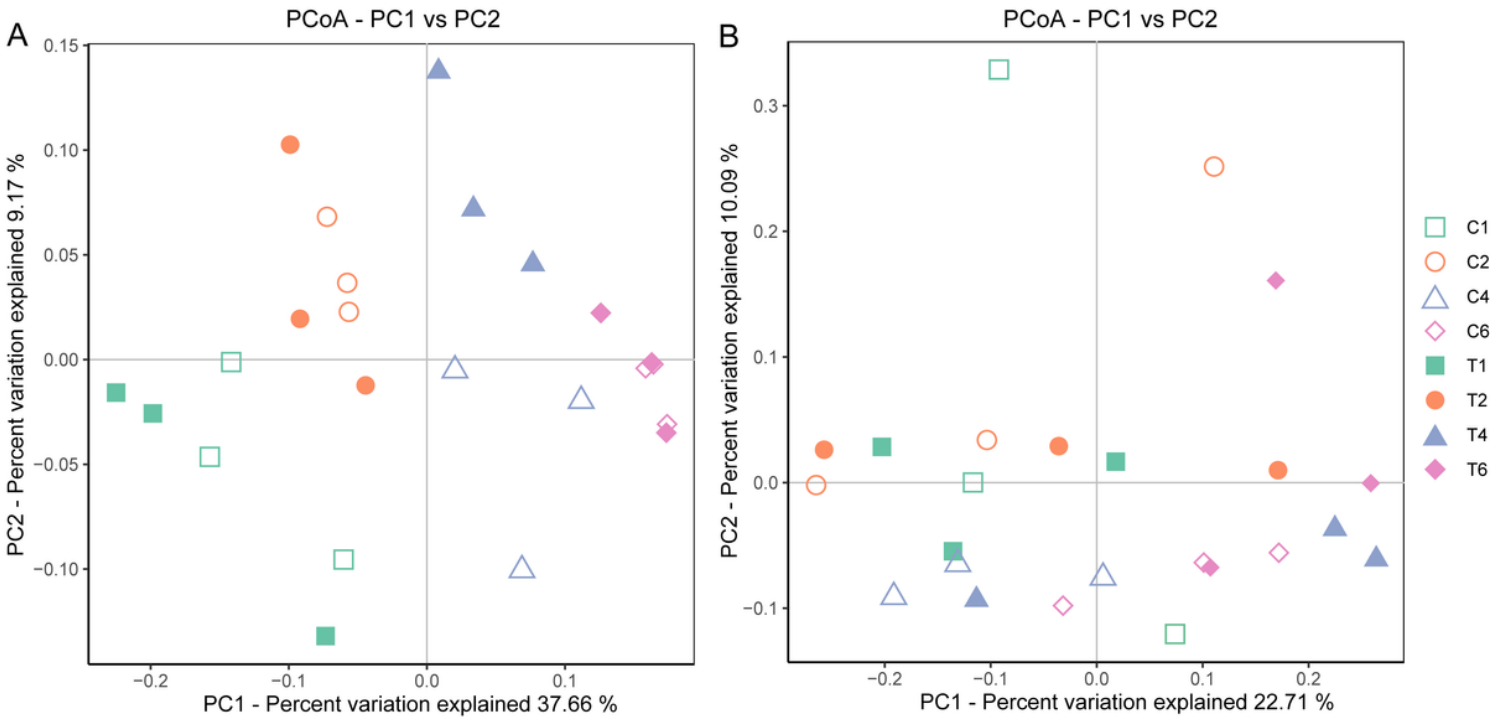

Figure 5

β-diversity of soil bacteria and fungi based on PCoA (Principal coordinate analysis) using Bray–Curtis dissimilarities at six-month degradation stages between the EB treatment and control. (A) β-diversity of soil bacteria analysis based on PCoA; (B) β-diversity of soil fungi analysis based on PCoA.

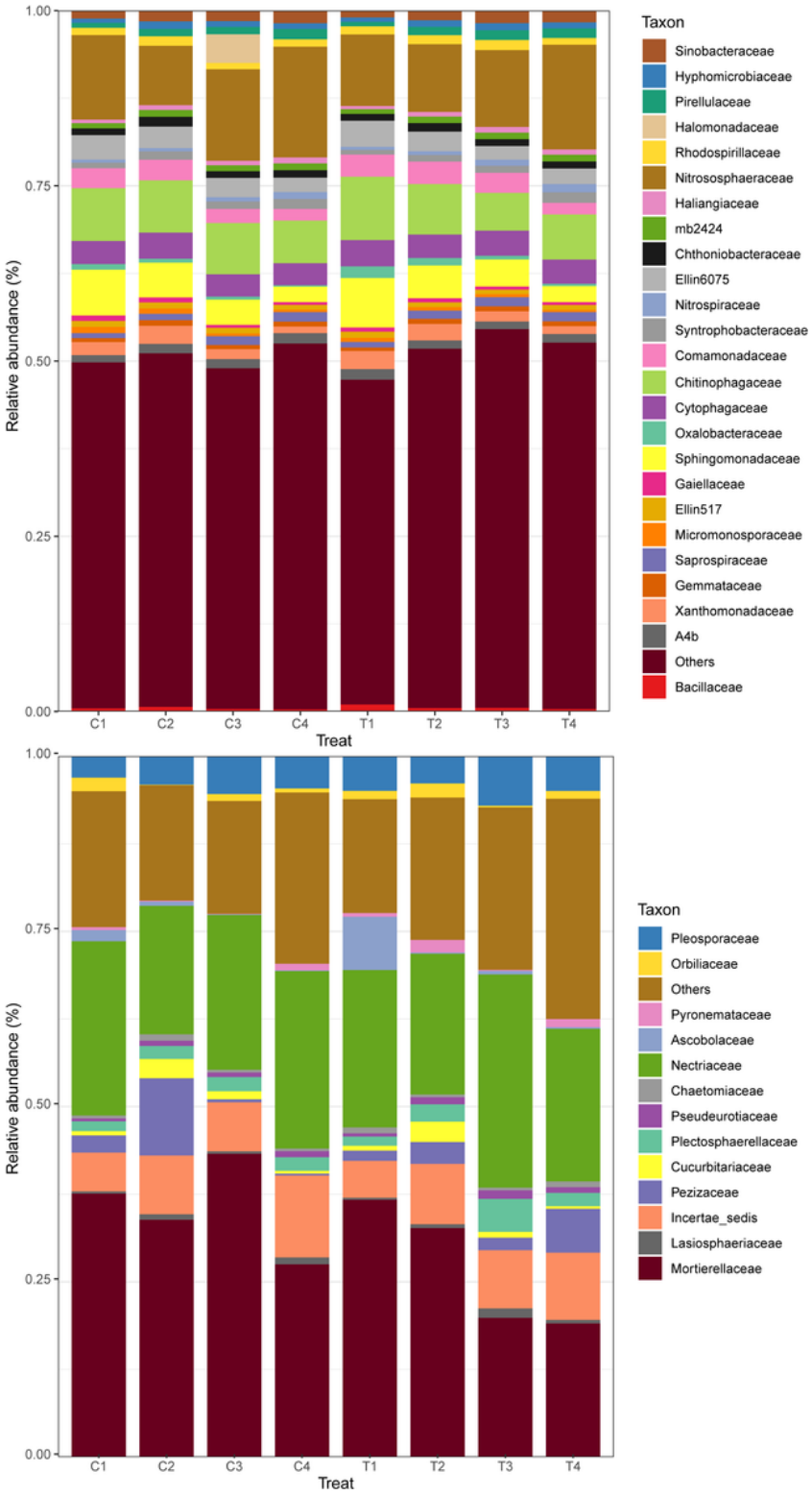

Figure 6

Relative abundance at the family level of dominant soil bacteria and fungi at four harvested months (1st, 2nd, 4th and 6th month) over six months of decomposition of A. thaliana litter. C = control, T = harvest time of soil sample in EB treatment soil; different colors indicate different families of bacteria and fungi, different lengths represent relative abundance; (A) relative abundance of bacteria, (B) relative abundance of fungi.

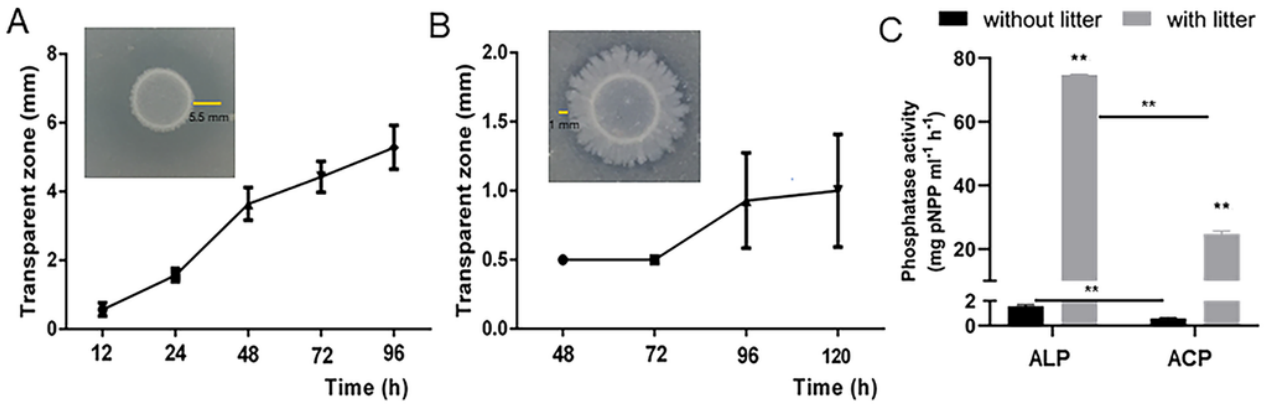

Figure 7

Activity of EB phosphate solubilization and phosphatase production. (A) organophosphorus degradation cycle; (B) inorganic phosphorus dissolution cycle; (C) activity of alkaline and acid phosphatase production, ALP = alkaline phosphatase, ACP = acid phosphatase, "**" denotes significant differences ($P < 0.01$).

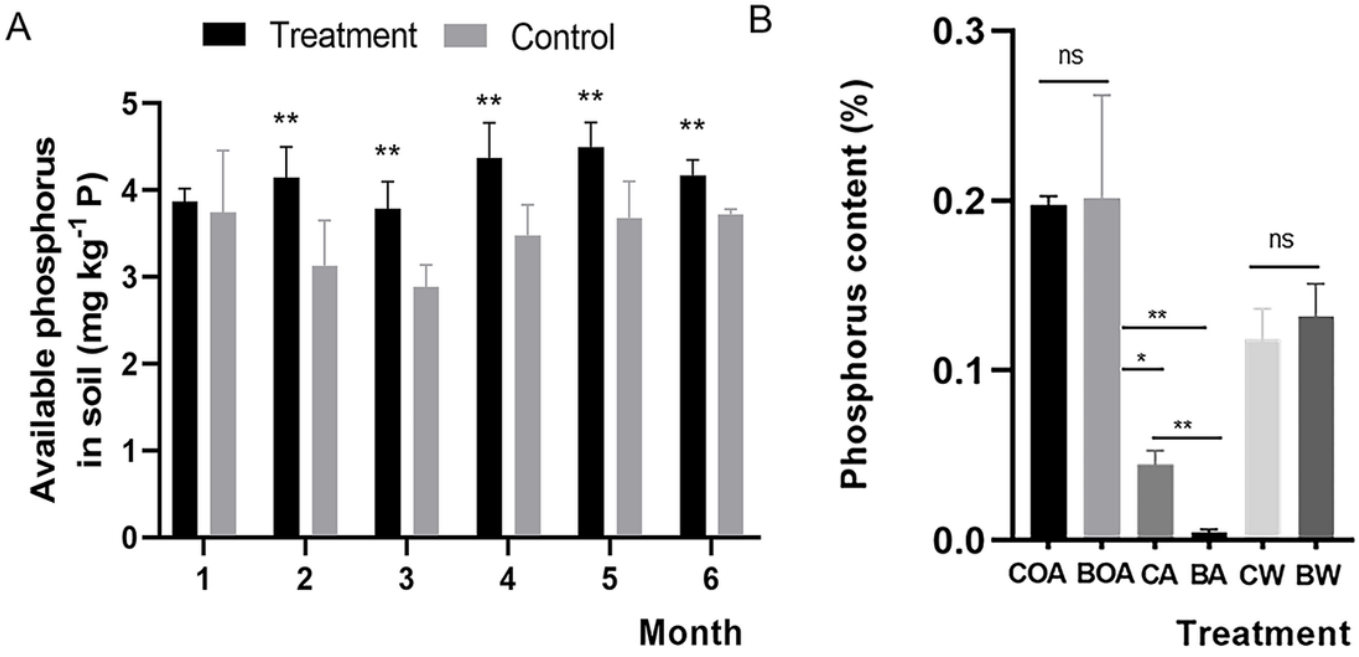

Figure 8

Changes in available phosphorus in soil and total P in plants during six months of A. thaliana litter decomposition. Error bars indicate standard deviation, "*" denotes significant differences ($P < 0.05$), "**" denotes significant differences ($P < 0.01$), "ns" indicates no significant difference ($P > 0.05$). (A) Available

P in soil over six months of decomposition; (B) Total P content in plant, COA = control without EB from original A. thaliana litter, BOA = EB pre-inoculated original A. thaliana litter, CA = control of A. thaliana litter after six months of decomposition, BA = EB released from A. thaliana litter after six months of decomposition, CW = wheat planted in six-month decomposition control soil, BW = wheat planted in six-month decomposition EB treatment soil.

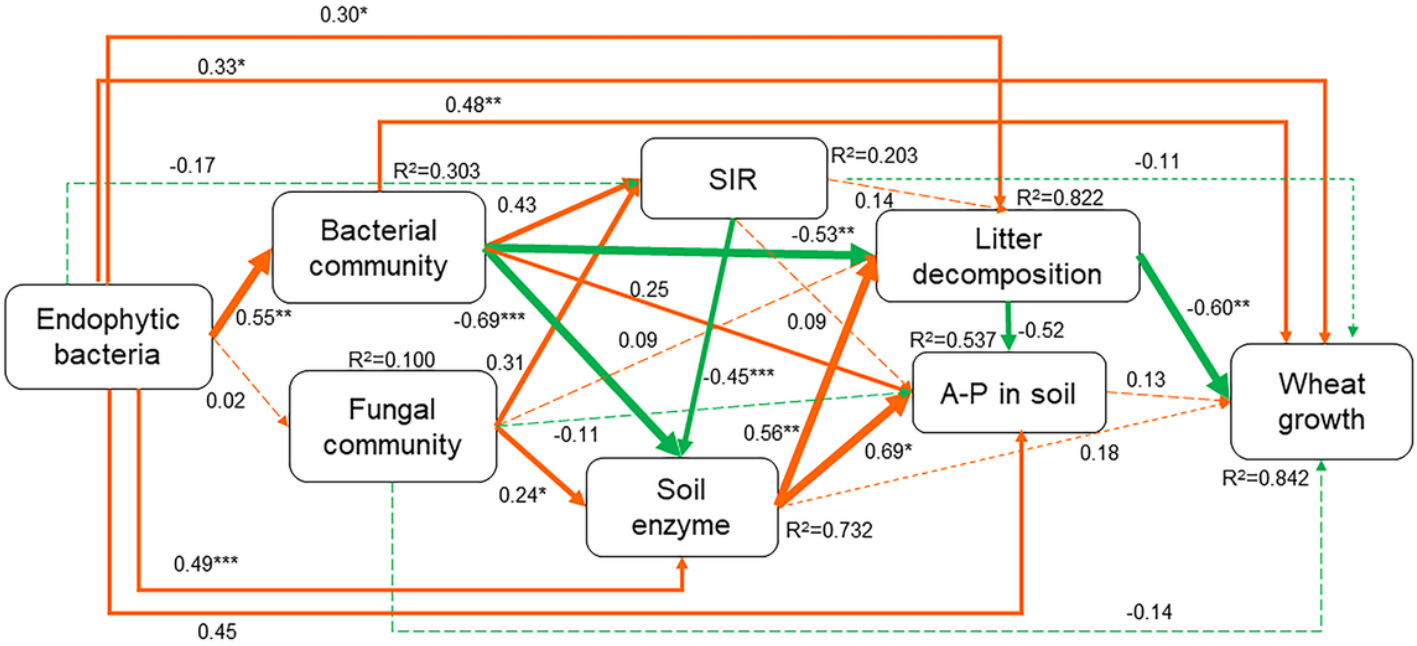

Figure 9

Structural equation model showing the direct and indirect interactions between endophytic bacteria, bacterial and fungal richness in soil, SIR, soil enzyme activity, litter decomposition, available P in soil and wheat growth. The width of arrows is proportional to the strength of path coefficients. Orange and green arrows indicate positive and negative flows of causality, respectively. Numbers on each arrow indicate significant standardized path coefficients. Chi-square = 1.52 (P = 0.468); Root mean square error of approximation (RMSEA) < 0.001; standardized root mean square residual (SRMR) = 0.054; comparative fit index (CFI) = 1. *** denotes P ≤0.001; ** denotes P ≤ 0.01; * denotes P ≤ 0.05.

Supplementary Files

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