Original article

Soil microorganisms interacting with residue-derived allelochemicals effects on seed germination

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ABSTRACT

Despite the knowledge regarding allelopathy, known as a major ecological mechanism for biological weed control, had increased greatly, the role of soil microorganisms in that field remained controversial. The study sought to evaluate the interference potential of soil microorganisms, residues-derived allelochemicals and their interaction on seed germination and understand the variation of microbial community in allelopathic activities. Three different rice residues-derived fractions from variety PI312777 (extracts, straw fraction and fresh residue) were applied to sterile and live soils to disentangle the interference potential of soil microorganisms, residues-derived allelochemicals and their interaction concerned allelopathic activities. The results demonstrated that microbe-only and residues-only exerted onefold promotion and inhibition effects on lettuce (Lactuca sativa Linn.) seed germination, respectively, whereas, microbe-by-residues interaction showed an inhibition at the beginning, and a feeble promotion later. The 20 most dominant genera of microbes were classified into three clusters, with 13 genera in one cluster, only 1 in the second cluster and 6 in the third one. The genera in the first cluster commonly exerted negative effects on phenol content, while showed positive correlation with seed germination. Interestingly, Bacillus, clustered in the second cluster, had an opposite effect alone. The third cluster genera somehow had a weak correlation with both germination as well as the release of the allelochemicals. Overall, we incorporated molecular methodology for tracking bacterial impacts during incubation with allelochemicals, and demonstrated the mutable role of soil microbes in allelopathy. It may be potentially important for stimulating the beneficial roles of microbes for environmentally friendly weed management.

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1. Introduction

Biological control of weeds is widely accepted as a natural way towards weed management in agricultural systems due to the chemical herbicide pollution and increased emergence of herbicide-resistant weeds from the repeated use of synthetic herbicides (Hunt et al., 2017; Ojija et al., 2019; Xiao et al., 2017). Rotation involving cover crops is the most common tactic in weed control (Hunt et al., 2017; Gerhards and Schappert, 2020). Cover crops suppress the growth of neighboring plants through physical, chemical, and biological suppression, i.e., the competition of resource and light, disruption of weed life cycles, and primarily, releasing allelochemicals (Xiao et al., 2019). Once released into the soil, these allelochemicals affect the soil environment, and allelopathic activities occur simultaneously and continuously, subsequently influencing the target plant germination and seedling establishment (Xu et al., 2019; Gerhards and Schappert, 2020).

Allelopathy has gained extensive attention in biological weed control recently (Hunt et al., 2017). Moreover, allelopathy has been reported as a mechanism of invasive success, termed the Novel Weapons Hypothesis (Gerhards and Schappert, 2020). The allelochemical effects of inter- or intraspecific species primarily caused by the action of allelochemicals, which are mainly secondary metabolites known for their allelopathic potential (Pergo et al., 2008). For example, phenolics, released by a wide variety of plants, can reduce seedling growth, and flavones biosynthesized by higher

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plants can exert allelopathic effects on the rhizosphere (Levin, 1971).

Previous studies reported that the soil matrix could reduce phytotoxicity through a combination of degradative and adsorptive processes (Inderjit, 2005; Jilani et al., 2008; Lou et al., 2016), suggesting that the potential of allelochemicals is usually associated with their existence forms. Allelochemicals in the natural environment are composed of water-soluble fractions and insoluble fractions, which are bound up in straw residues, released by microbial decomposition as well as chemical decomposition in interactions between allelochemicals and subsequent microbial transformation of these chemicals (Lou et al., 2016; Barnes and Putnam, 1986; Inderjit, 2005; Ojija et al., 2019).

One of the fascinating but controversial processes is the role of microbes in allelopathy encompasses (Inderjit, 2005). The period of growth suppression potential was documented as the "window" by Lou et al. (2016), and biotic or abiotic factors in soil, e.g., microorganisms and allelochemicals, could widen or narrow the "window" to exert interference potential. Factors that inhibit the seed germination (allelochemicals release and transformation of harmless compounds to toxic forms) were regarded as synergistic with cover crop-derived allelochemicals, otherwise it is antagonistic. Microorganisms can moderate the releasing rate of allelochemicals, and hence affect seed suppression. Interestingly, microbes can affect these allelochemicals with both positive and negative outcomes (Lou et al., 2016). Microbes reportedly can play a negative role in allelopathic effects by deactivating water-soluble phytotoxins or decomposing toxic compounds to reduce allelopathic effects (Jilani et al., 2008). On the other hand, they can also release the insoluble phytotoxins bound up in stubborn fractions (Barnes and Putnam, 1986), and transform the harmless compounds to phytotoxins, subsequently exhibiting a synergistic effect with allelochemicals (Lou et al., 2016). The microbe-by-allelochemical interactions are so complicated and poorly understood that the role of microorganisms in allelopathy encompasses has remained equivocal. Thus, incorporating up-to-date molecular methodology for tracking bacterial impacts on allelochemicals is clearly needed.

Allelochemicals in the natural environment are composed of water-soluble fractions and insoluble fractions, which are bound up in straw residues released by microbial decomposition as well as chemical decomposition (Inderjit, 2005; Barnes and Putnam, 1986). Lou et al. (2016) designed a method to characterize the contributions of two sources allelochemicals of cover crop residues. Here, we mirrored separation of soluble and insoluble fractions, and modified evaluation procedure of Liebman and Sundberg (2006), striving to make a case for extending the investigation to the rice systems and gain a deep insight into the interaction between soil microbial communities and allelochemicals.

Therefore, this study aimed to (i) characterize the allelopathic potential of rice PI312777 residues-derived fractions; (ii) understand the variation of microbial communities in allelopathic activities concerned; and (iii) evaluate the interference potential of soil microorganisms, residues-derived allelochemicals and their interaction on seed germination, in order to advance from simplistic laboratory demonstrations of allelopathic potential to useful evidence of allelopathic weed suppression in fields.

2. Materials and methods

2.1. Soil samples

We chose a field that had not grown any crop plants since 2013 at Zhejiang, China (30°4′49″N, 119°56′1″E). Soils were collected from a top 15-cm layer. The soil was Typic Epiaqualf (16.78% clay; 40.62% silt; 42.60% sand; pH 6.47). Soil samples were divided into 2 portions: one was autoclaved three successive times (103 kPa, 120 °C, 1 h) to establish the role of soil microorganisms in allelopathy, and the other remained unchanged.

2.2. Plant materials

Recognized allelopathic rice (*Oryza sativa* Linn.) cultivar PI312777 was employed in this study. PI312777 residues were provided by Professor Lu Yongliang of China National Rice Research Institute and field-collected at ripening stage. Lettuce was employed as an observable indicator due to its better sensitivity for allelochemicals and is widely used as an assay species (Xiao et al., 2017).

2.3. Experimental design of residue incubation and bioassay approach

The fresh rice stems and leaves of PI312777 were cut into 2 cm pieces, and one portion was processed to water-soluble extracts and straw fractions to evaluate the allelopathic potential of soluble and insoluble compounds, respectively (Lou et al., 2016). And the remained portion was attempted to estimate the fresh residue-driven allelopathic effects on seed.

Residues (30 g) destined for the soluble and insoluble components treatments were agitated twice in 300 ml deionized water for 10 h at 25 °C. The remaining mixture was then separated into residue and liquid fractions referred to as the straw fractions and water-soluble fractions, respectively. The straw fraction and fresh residues were exposed to UV light for 3 h on each side prior to incubation. The liquid fractions were centrifuged (3500g, 15 min) to recover water-soluble allelochemicals and finally concentrated six-fold by freeze-drying to 100 ml. Sterilization by filtration, a regular method, for water extracts was used. The extracts were filtered twice using 0.22 μm microporous membrane filter. 1% (m/m) PI312777 residues were sufficient to elicit an allelopathy response in our preparatory experiment (data are not shown). Due to the separation of water-soluble (extracts) and insoluble allelochemicals (straw fraction), here, 2% PI312777 residues or the equivalent amount of potentially bioactive compounds found in 2% PI312777 residues were applied. 150 g of sterile or live soil amended with 3 g fresh residues was placed in pots, and the straw fraction or the extracts of 30 g residues (see above) were divided into 10 parts and then applied into soil. The absence of residue treatments served as controls (applied with 10 ml distilled water), and then the pots were conducted for 0, 1, 2, 4, 8 and 16 days of residue incubation. Thus, a total of 144 pots (two microbes × four treatments × six time points × three replicates) were arranged in an incubator with a constant humidity of 75% at 25 ± 2 °C.

At each time point, 24 pots (two microbes × four residue treatments × three replicates) were conducted the bioassay approach. The rolled-towel cold-test method with minor modifications (described below), designed initially to assay the effect of cover crops, was used to assess the allelochemicals and microbial impact on seed germination (Hoppe, 1955). 50 g of soil from above-mentioned incubation pots were spread in the 12 cm-wide band, a double layer of germination paper (37 × 25 cm), and was moistened with 20 ml water. Then, 15 pre-sterilized lettuce seeds were placed in a 30 cm-long line about 10 cm from the top edge of the germination paper. Another 50 g of incubation soil was distributed to cover the seeds either top-placed or mixed with this soil portion. Another sheet of moistened paper was placed on top of the soil, and the entire assembly was rolled to form a cylinder. These cylinders were wrapped and sealed to minimize the exchange of volatiles and incubated vertically with seeds near the top, at 25 ± 2 °C under 8 h dark-16 h light cycle. We had previously reported that lettuce seed germinated on day 2 (Xiao

et al., 2017), in this study, we carried bioassay experiments for 5 days, which was long enough to determine if cumulative germination eventually reached control levels. Even though the root length inhibition was often the most sensitive indicator of allelopathy in early growth, we measured the germination of lettuce seeds as an indicator to distinguish the interference potential of microorganisms and residues on seedling growth (described below).

2.4. Total phenolic compound extraction and quantification

Total phenolic compounds originally contained in or applied to the soil were extracted from 5 g freeze-dried soil using 2 × 15 ml of an organic extractant solution (acetonitrile/methanol/acetone 25:70:5). The suspensions were then shaken mechanically for 5 h and centrifuged at 3500g for 15 min and the supernatant retained. The supernatant fractions were concentrated to 2 ml with N_2 at 37 °C. Quantification of total phenolic compounds was conducted by the Folin-Ciocalteu method (Lou et al., 2016).

2.5. DNA extraction and quantification of microbial abundance

16S rDNA sequence analysis was conducted to estimate the microbial community changes. Genomic DNA was extracted from 0.5 g of a soil sample from the beginning of each bioassay using the Rapid Bacterial Genomic DNA Isolation Kit (Sangon Biotech, China). Polymerase chain reaction (PCR) amplifications were conducted using the universal bacterial primers 341F (5′-CCTACGGG NGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) to target the conserved V3 and V4 regions of the 16S rRNA gene.

2.6. Statistical analysis

We quantified the interference potential (IG) of microorganisms and residues on germination using the procedure of Liebman and Sundberg (2006), with minor modifications. The interference could be partitioned into three sources: microbe-only (IGM), residue-only (IGR), microbe-by-residue interaction(IGI) (Lou et al., 2016). In order to compare different sets of treatments, we supposed that the microbe and residues were additions to sterile soil receiving water-only. Thus, the interference potential of microbe and residues were estimated by comparing soil with treatments and sterilized soil with water-only. The calculations were modified without hypothesizing whether the role of microbes was promotive or suppressive to germination in advance, and were illustrated in Fig. 2. The positive value (>0) of interference potential means promotion to germination, and the negative value (<0) indicates suppression.

We processed the pyrosequencing data using the Qiime 2 pipeline. Redundancy analysis (RDA) was used to evaluate the relationship of response variable values (20 most abundant bacteria genera) and environmental variables (total phenolic content and germination rate), and carried out by Canoco for windows 4.5. Statistical analysis was performed using SPSS 22 for Windows. ANOVA were used to analyze the significance level (P < 0.05).

3. Results

3.1. Soil microorganisms regulated lettuce seed germination dramatically

The germination rates of lettuce seeds differed distinctly or sometimes significantly with respect to the presence or absence of the microbial community (Fig. 1). When treated with water-only, the germination rates in live soil treatments were significantly higher (approximately > 10%) than those of in sterilized soil ones (Fig. 1a). In the treatments of soil with water-soluble extracts,

however, the seed germination rates fell deeply compared with control treatments whether in live or sterilized soils. There were no significant differences in seed germination rates between live and sterilized soils on days 1, 2, and 4, but the values of germination increased rapidly from day 8 forward, and became significant differences between various soil treatments (Fig. 1b). A similar trend could be observed in soils with fresh residues and water-soluble fractions, although the former showed a convergent trend on day 16 (Fig. 1d). Interestingly, when soils treated with straw residues, their seed germination rates almost reversed compared with above three ones due to the interference of microbial community (Fig. 1c). The absence of a microbial community in sterile soils allowed for at least twice the amount of seed germination than live soils, even four times on day 2, though the treatments of the live soils surpassed the sterile soil ones on day 16 (Fig. 1c).

3.2. Interference potential of soil microorganisms and residue-derived fractions

The interference potential of microbe-only, residues-only and microbe-by-residue interaction to lettuce seed germination varied dynamically (Fig. 2). The microbe-only showed contributing to the establishment of seed germination, whereas residues-only fractions (extracts, straw fraction and fresh residues) had negative values, showing germination inhibition (Fig. 2) that decreased in the order: fresh residues > water extract > straw residues. However, the interference of microbe-by-residue interaction shifted from an early significant inhibition phase to a later feeble influence or promotion phase (Fig. 2b–d).

3.3. Total phenol content

The total phenol concentration of different fractions in soil generally decreased in the order: fresh residues (4.07–13.32 ng/g soil) > water extracts (2.38–8.66 ng/g soil) > straw residues (2.27–6.09 ng/g soil) > background soil (0.26–3.60 ng/g soil). A rise-fall pattern in straw fraction treatments and a fall-rise-fall pattern of total phenol concentration in both water-soluble and fresh residue treatments were observed (Fig. 3). Besides the water extract treatments, the presence of soil microorganisms made live soil have higher phenol concentration than sterile soil.

3.4. Variation of microbial communities

16S rRNA sequencing detected a total of 28 phylotypes, but only 6 dominant phyla in initially sterile soil (Fig. 3b, c and d) were found. Particularly, Proteobacteria accounted for a major proportion (91.9% ± 2.4%) (Fig. 4). Firmicutes and Actinobacteria increased dramatically on days 1 and 2 but declined in the later stages. Proteobacteria and Actinobacteria in live soil generally increased compared to the control (Fig. 4). Notably, Actinobacteria was the dominant phylotype in live soil, even reaching 63.3% on day 8, which was twice more than that of the control. Whether the species richness or diversity of the microbial community was, the live soil was usually higher than sterilized soil, and showed a similar tendency: a rise in the first two days, followed by a decline from days 2 to 8, occurred in the highest level on day 16 (Fig. 5). The similar trend of Actinobacteria and ACE Estimator Richness indicated that Actinobacteria was probably the major contributors to the changes in alpha diversity (Figs. 4 and 5).

At genera level, the variation of 3 most abundant bacterial genera in sterile and live soil had illustrated (Fig. 6). Chitinophaga (Sphingobacteriia) was barely detectable in the early stage (days 1 and 2) but increased dramatically on day 4, and thereafter remained at a relatively high level. The abundance of Enterobacter and Pseudomonas (both γ-Proteobacteria) dramatically reduced on
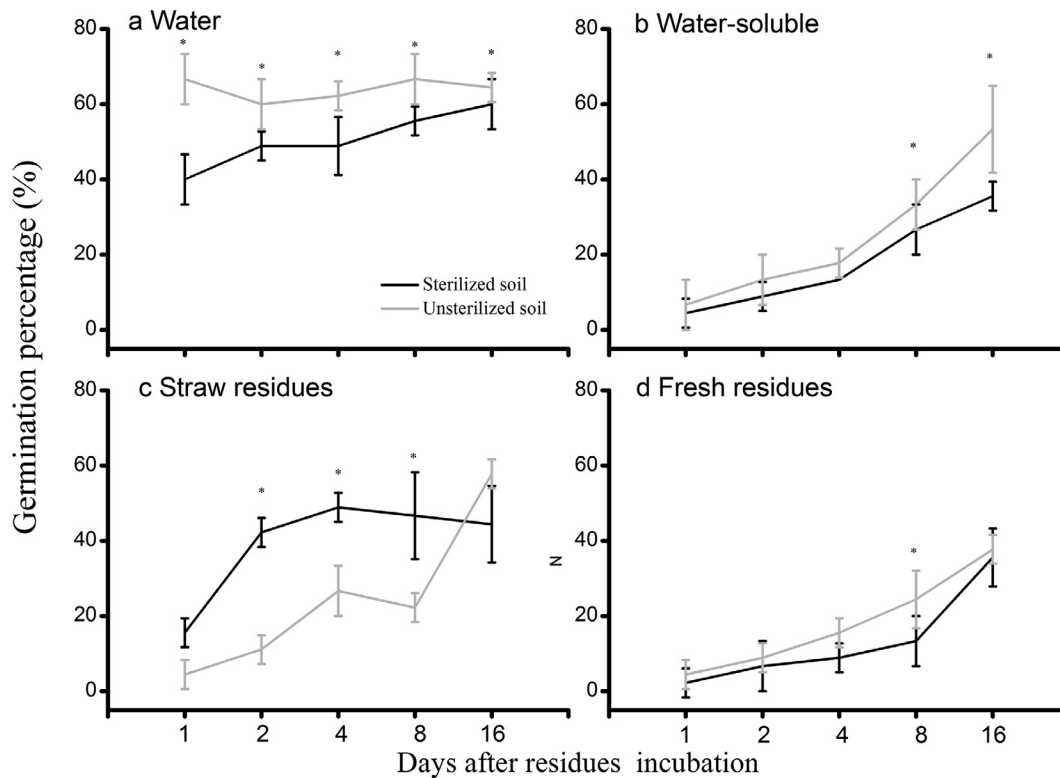
Fig. 1. Germination rates of lettuce seeds differed distinctly in treatments with different application. Percentage of seed germination in sterilized and live soil were shown for treatments exposed to (a) water, (b) water-soluble extracts, (c) straw residues, and (d) fresh residues. Mean ± SE from three replicate experiments is shown. Asterisk indicates comparisons that were determined to be significantly different at p < 0.05.

day 1, and exhibited a relatively strong or weak rebound in the later stage, respectively. In live soil, the abundant bacterial genera *Streptomyces* (*Actinobacteria*) and *Bacillus* (*Bacilli*) represented two patterns of change (Fig. 6b), which were composed of two stages according to their relative abundance. A low richness of *Streptomyces* in the first stage (days 1 and 2) was followed by a high abundance on days 4, 8, and 16. The reverse pattern for *Bacillus* was observed, and *Nocardioides* (*Actinobacteria*) showed a stable pattern throughout the experimental process.

4. Discussion

With the addition of residue-derived fractions (extracts, straw fraction and fresh residue), the treatments had higher base level of phenolics and lower lettuce seed germination than background soil, suggesting that all three residue fractions contained allelochemicals and influence the germination of lettuce, to some extent (Figs. 1–3). It had been described that the allelochemical effects of plant residues were mostly due to water-soluble allelochemicals (Barnes and Putnam, 1986). As demonstrated in Fig. 1, the patterns of seed germination rates in treatments were in agreement with literature data, and similar results were also obtained from the total phenolic levels (Fig. 3). Once water extracts and fresh residues were applied to soil, the content of water-soluble allelochemicals in soil decreased rapidly due to their adsorption, decomposition and microbial degradation in soil (Lou et al., 2016). These processes caused a fall in phenol concentrations in the initial period (Fig. 3b and 3d), which was absent in straw residue treatments due to the lack of water-soluble allelochemicals (Fig. 3c).

The combination of residue-derived fractions with soil microorganism exhibited much-complicated effects on phenolics. The promotive effects of microbes in straw residue and fresh residue treatments were observed. The presence of soil microorganisms

in live soil led to higher phenol concentration than sterile soil with similar treatments, but a reverse pattern in water extract ones suggested that microbes played opposite impacts on water-soluble allelochemicals (Fig. 3). It is probable that rice residue was mostly composed of insoluble allelochemicals, and microbes in live soil released the insoluble allelochemicals bound up in the recalcitrant fractions, leading to a higher phenol concentration in live soil than in sterile soil (Fig. 3c and d). Additionally, it has been documented that solid residue can prolong the interaction phase by acting as reservoirs for both soluble and insoluble allelochemicals, protecting the allelochemicals from microbial attack (Lou et al., 2016). In water extract treatments, the exposure of water-soluble allelochemicals to microbes and lack of water-insoluble allelochemicals resulted in a lower phenol level in soil with a live microbial community (Fig. 3b).

As discussed above, it was discovered that microbes exerted inconsistent interference on allelochemicals and seed germination in soil with different application (Figs. 1–3). In fact, the amphibious role of soil microorganisms had been frequently reported. Li et al. (2015) reported that live soil reduced the allelopathic effect of leaf leachate of 8/9 plant species, showing a promotive effect on plant growth. Diametrically, the microorganisms were documented inhibitory effects on seedling growth when treated with red clover residues (Inderjit and Foy, 1999). The inconsistent results indicated the amphibious role of soil microorganisms. Regardless of the microbial degradation of allelochemicals in extracts treatments or phenol release in residues treatments, it is reasonable to believe that the soil microbes played an invaluable role to affect the phenol levels and moderate the allelopathic activities at significant rates (Figs. 1–3).

The microbe-only showed contributing to the establishment of seed germination, whereas the interference potential of residues-only (extracts, straw fraction and fresh residues) had the negative
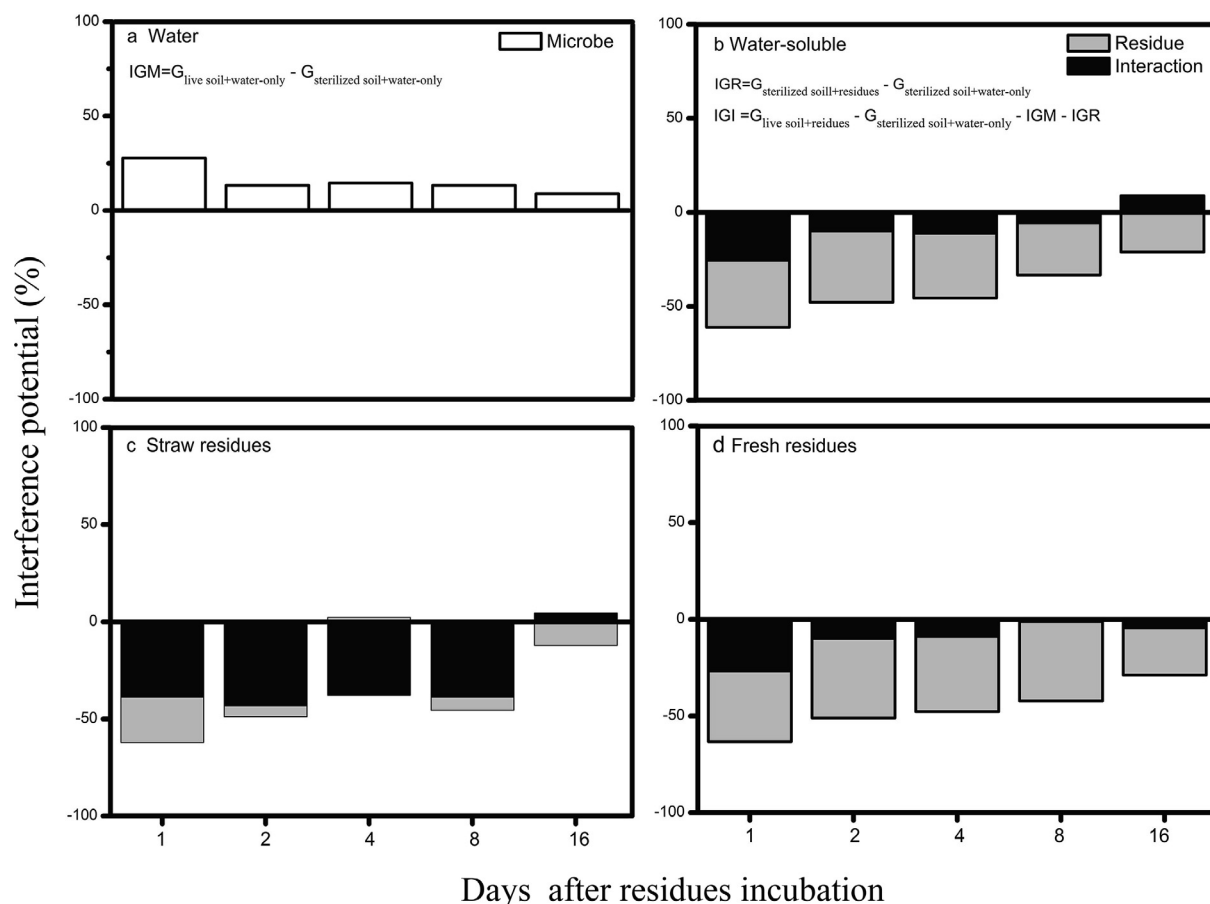
Fig. 2. The interference potential of soil microorganisms, residues-derived fractions and their interaction on seed germination. (a) water, (b) water-soluble extracts, (c) straw residues, and (d) fresh residues. Mean from three replicate experiments is shown. The positive value (>0) of interference potential means promotion to germination, and the negative value (<0) indicates suppression.

value, showing germination inhibition (Fig. 2). Whereas, microbe-by-residue interaction showed a mutable role in seedling growth: significant inhibition falls in water-soluble and fresh residue treatments over time, a relatively stable inhibition in soils with straw fraction, but a feeble influence, sometimes, even a promotion in the later periods (Fig. 2), indicating a mutable role of soil microorganisms over time, indeed (Inderjit, 2005). In the prophase, the inputs of residue stimulated microbial activities, resulting in microbial release or transformation of allelochemicals contained in residues (extracts). Associated with adsorption and decomposition, these processes caused an inhibition fall in the initial period (Fig. 2). With residues consumed over time, microorganisms, subsequently, decomposed the metabolites that persisted in the soil (Inderjit, 2005), leading to a feeble influence even a promotion on germination in a later period. Thus, the rise-fall pattern in phenol levels (Fig. 3b–d) and dynamics in interference potential of microbe-by-residue interaction were showed (Fig. 2).

It is tempting to speculate that there were particular allelochemicals and specific microflora in different stages in the variation of microbial communities. In these processes, the microflora was influenced by allelochemicals, and soil microorganisms regulated allelochemicals in turn (Inderjit, 2005; Xiao et al., 2017), which were exemplified in numerous studies. *Streptomyces* was closely related to the metabolism of ferulic acids and cinnamic, and *Acinetobacter* could be mediated by 2,3-benzoxazolinone (Sutherland et al., 1983). We had strived to isolate particular allelochemicals and quantified the extraction using LC-MS but failed, due to the extraction limitations in the soil matrix. It had been

documented that allelopathic rice contained a range of phenolic acids (e.g., vanillic, *p*-hydroxybenzoic and ferulic acids), a few flavones and diterpenoids (Kong et al., 2008). Given the established components of PI312777, we quantitated the total phenol content instead. Furthermore, microflora, a vital indicator of allelopathy (Inderjit, 2005), was characterized. The available data revealed that completely sterilize soils was difficult and the microbial communities recovered rapidly in a short time, such as 2 days in this case (Figs. 4–6). We also had used a mild method to obtain sterile soil, such as exposure to UV light, and obtained soil with poor sterilization effectiveness in our preparatory experiment.

According to the subtle changes of alpha diversity (tendency), key phyla (e.g., *Firmicutes* and *Actinobacteria*) and genera (e.g., *Chitinophaga* and *Bacillus*), we divided the microbe variation into three phases. Initially, there was an early selective kind of stimulation phase, then a selective kind of inhibition phase appeared, and lastly a late recovery phase (Figs. 4–6). The addition of residues (extracts) inhibited or stimulated somewhat specific microbial populations in soil, leading to the variation of microbial communities based on the soil indigenous microbial communities (Cipollini et al., 2012). Moreover, the similar substrate modification input might cause different direction and degree of response of microbial activities (Kong et al., 2008), rustling in the different dominant genera in soil with various treatments (Figs. 4–6).

The relationships of 20 most abundant bacteria genera, total phenol content, and germination were illuminated by redundancy analysis (RDA) in Fig. 7. Unsurprisingly, the germination rate had a significant negative correlation with total phenol concentration,
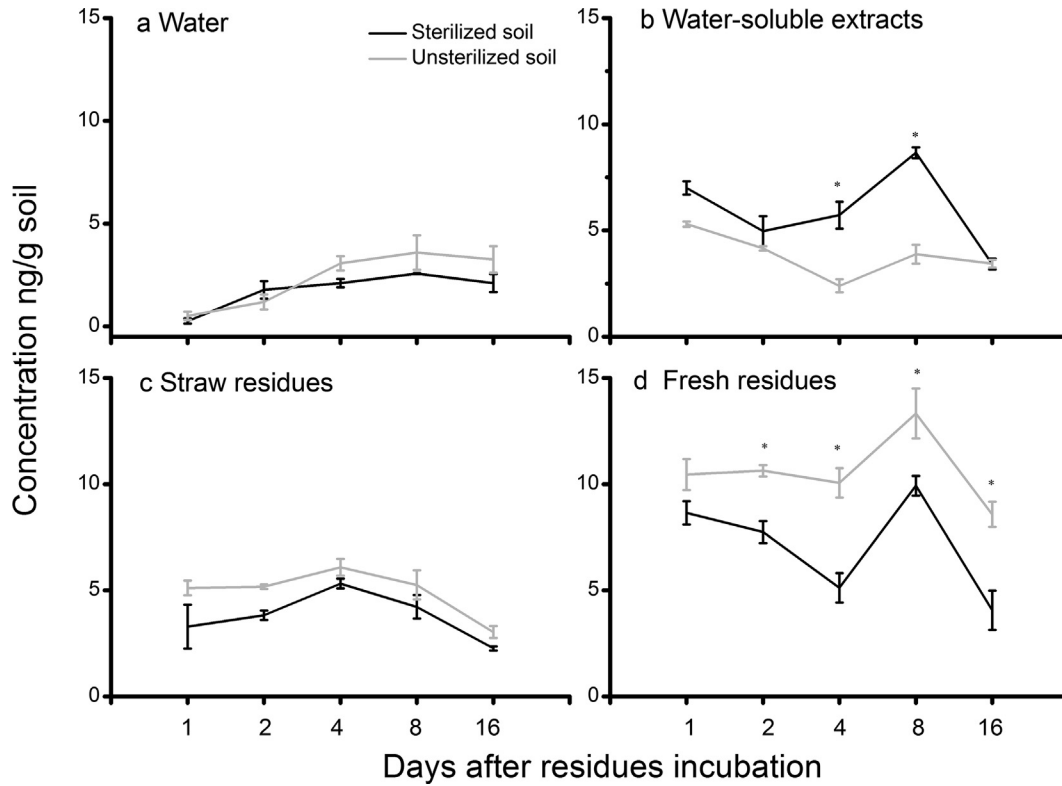
Fig. 3. Total soil phenol concentrations in sterilized and live soils varied in treatments with different application. (a) water, (b) water-soluble extracts, (c) straw residues, and (d) fresh residues. Mean ± SE from three replicate experiments is shown.
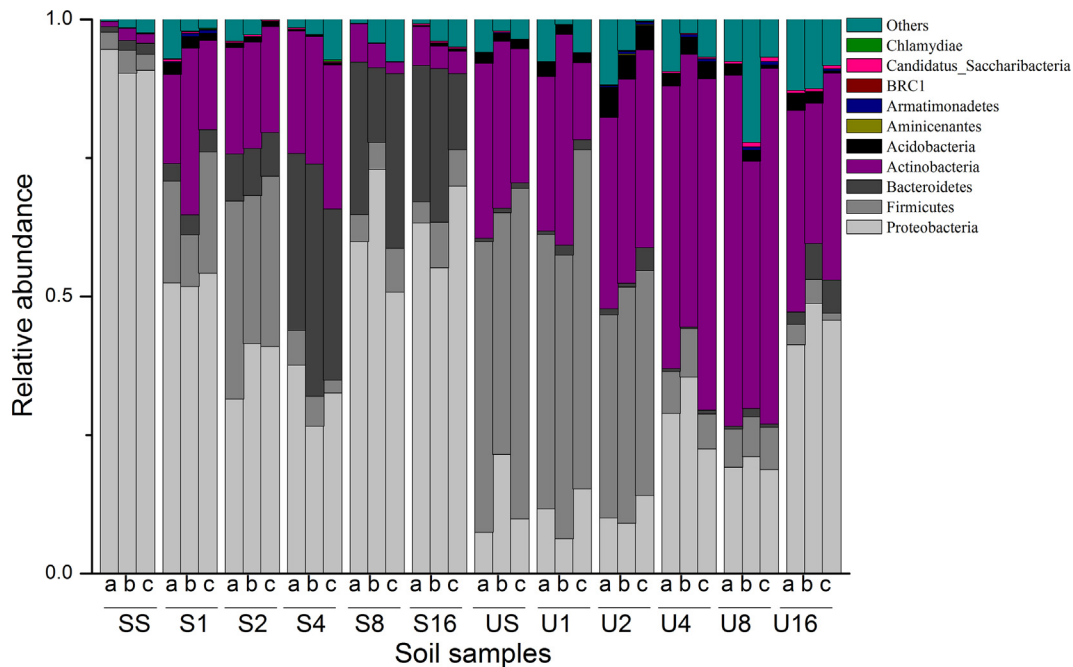

Fig. 4. Variation of dominant phyla of active bacterial community in soil with fresh residue over time. SS, initially sterile soil; S1–S16, sterile soil after 1–16 days residue incubation; US, initially unsterile soil (live soil); U1–16, live soil after 1–16 days residue incubation. Lowercase letter (a, b and c) indicate three replicate experiments.

and it had been widely accepted (Kong et al., 2008; Xiao et al., 2017). To exert allelopathic effects on receptors, the allelochemicals should persist in soil and accumulate at phytotoxic levels, which directly mediated its bioavailability (Inderjit, 2005; Xiao

et al., 2017). However, the relationships between microorganisms and total phenol content or germination varied in association with the genera. The 20 most dominant genera of microbes were classified into three clusters according to their effects on germination
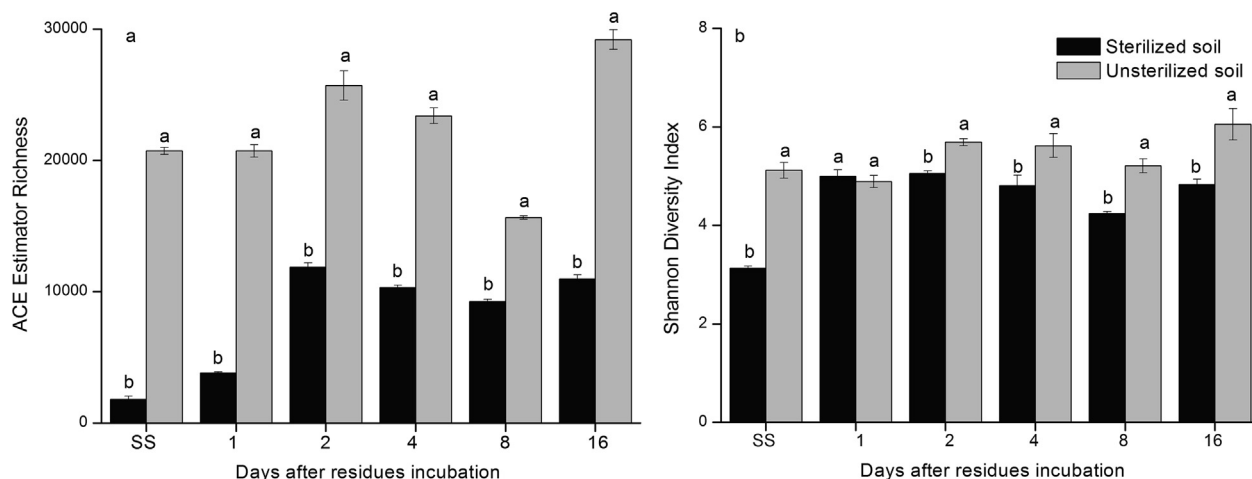
Fig. 5. Alpha diversity changes of active bacterial community in soil with fresh residue. (a) Abundance-based coverage estimate (ACE), (b) Shannon diversity index over time. Mean ± SE from three replicate experiments is shown. Columns with different letters indicate significant differences between individual treatments.
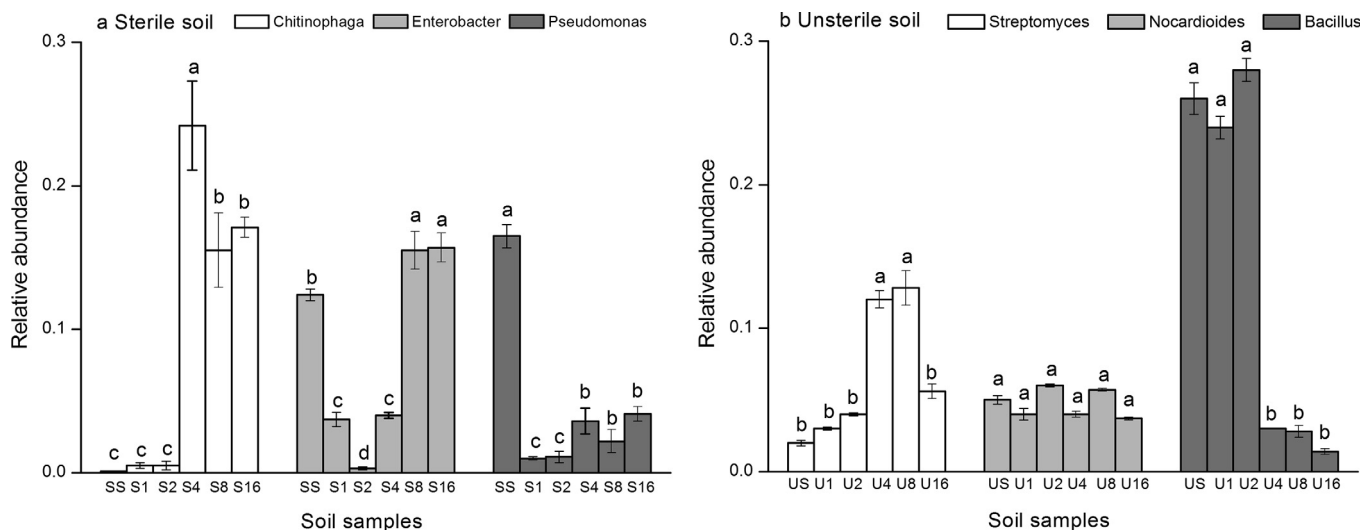

Fig. 6. Variation of the three most abundant bacterial genera in sterile and live soils over time. (a) sterile soil, (b) unsterile soil. Mean ± SE from three replicate experiments is shown.

and allelochemicals, with 13 genera in one cluster (indicated by the circle in Fig. 7), *Bacillus* alone clustered in the second group, and the rest being included in the third cluster.

Most dominant genera (13/20) exerted negative effects on total phenol content and showed positive correlation with seed germination (Fig. 7), which had been reported more frequently. Ehlers (2011) found that microbes could alleviate the phytotoxicology of thyme monoterpene in grass system, and Blum (1998) elucidated microorganisms could degrade phenolic acids and deactivate their phytotoxicity. Moreover, microbial community reportedly could utilize phenolic compounds as carbon sources (Inderjit, 2005), and *Pseudomonas* (γ-Proteobacteria) even took juglone as its sole carbon source (Schmidt, 1988). The microbial degradation of phytotoxins and decomposition of subsequent organics decreased the phytotoxicity and nourished the soil, benefiting the seedling growth. The third cluster of genera, such as *Enterobacter* (γ-Proteobacteria) and *Kitasatospora* (Actinobacteria), somehow

had a weak correlation with both germination and allelochemical release (Fig. 7).

Only *Bacillus* (Bacilli), clustered in the second cluster, showed a positive correlation with phenolic concentration but showed germination suppression (Fig. 7). It had been established that species of *Bacillus* were almost ubiquitous in nature and could occur in soil with extreme environments, and some species of *Bacillus* could be used as an insecticide, e.g., *B. thuringiensis*, which could release toxins to kill insects (Orhan et al., 2010). The unique effects and status in this study might result from its specialty. However, as far as we were aware, there are limited reports about the effect of *Bacillus* species on allelopathy research. Although a detailed investigation of toxins or allelochemicals produced by microbes is beyond the scope of this work, we acknowledge that future studies with a deep insight into allelochemical shifts and ecologically relevant interactions between allelochemicals and microflora are needed to enhance our understanding of allelopathy encompasses.
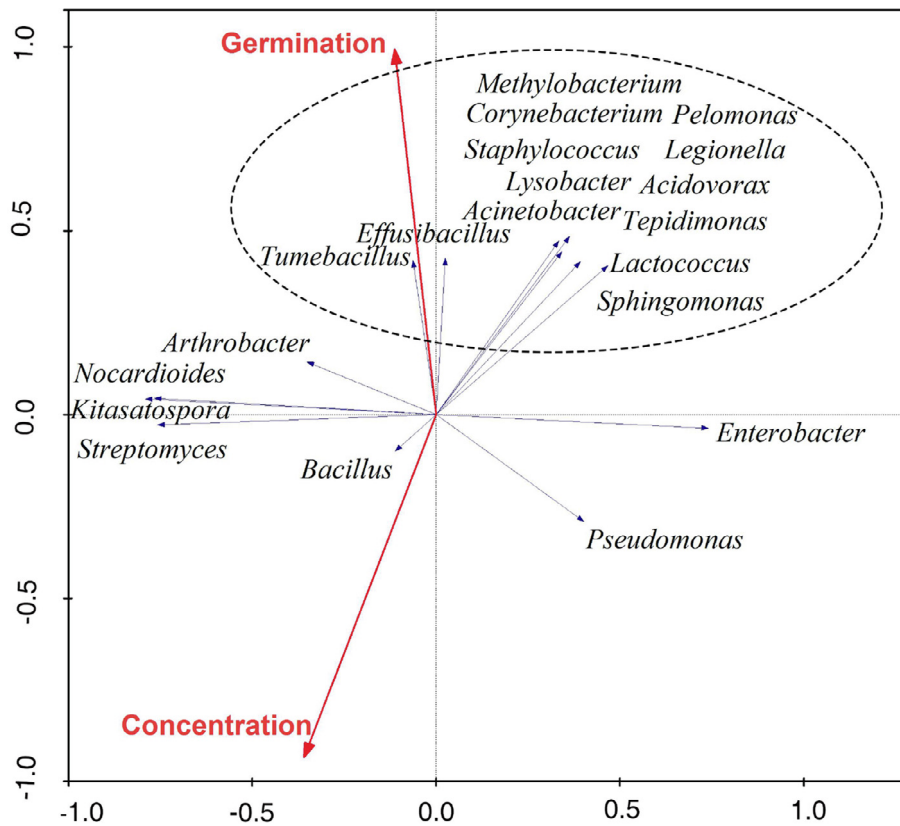
Fig. 7. Redundancy analysis (RDA) plot based on the dynamics of seed germination, phenol concentration and 20 most dominant bacteria genera.

5. Conclusions

In this study, the combination of sterilized or unsterilized soil with soluble or insoluble allelochemicals application provided a deep insight into microflora variation. Microbe could mediate allelochemicals release in soil and in turn, the addition of residues (extracts) inhibited or stimulated somewhat specific microbial populations, leading to the variation of microbial communities based on the soil indigenous microbial communities. Compared with the onefold promotion effect of microbe-only and inhibition of residues-only, the interference of microbe-by-residue interaction showed mutable role: the inhibitory effects got stronger firstly but fell over time, then a feeble influence even a promotion in the later period, indicating the mutable role of soil microorganisms over time, indeed. Most genera of microbes (13/20) were generally antagonistic with PI312777 residues (allelochemicals) and promoted seed germination, but a few microorganisms did the opposite, e.g., *Bacillus*. The available data called attention to the nature of the specific role of soil microorganisms in allelopathy encompasses. Clearly, the microbial activity is a double-edged sword in natural ecosystems, and a good understanding of beneficial roles of microbes may be potentially important for guiding development and understanding of modes of action of allelopathic cultivars for reducing economics of weed management that generally requires inputs of costly herbicides.

6. Compliance with ethics guidelines

Zhongxiang Xiao, Tao Zou, Shenggao Lu and Zhenghao Xu declare no competing interests.

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References

Barnes, J.P., Putnam, A.R., 1986. Evidence of allelopathy by residues and aqueous extracts of rye (*Secale cereale*). Weed Sci. 34, 384–390.

Blum, U., 1998. Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. J. Chem. Ecol. 24, 685–708.

Cipollini, D., Rigsby, C.M., Barto, E.K., 2012. Microbes as targets and mediators of allelopathy in plants. J. Chem. Ecol. 38, 714–727.

Ehlers, B.K., 2011. Soil microorganisms alleviate the allelochemical effects of a Thyme Monoterpene on the performance of an associated grass species. PLoS One 6, e26321.

Gerhards, R., Schappert, A., 2020.. Advancing cover cropping in temperate integrated weed management. Pest Manage. Sci. 76, 42–46.

Hoppe, P.E., 1955. Cold testing seed corn by rolled towed method. Wisc. Agric. Exp. Stn. Bull. 507.

Hunt, N.D., Hill, J.D., Liebman, M., 2017. Reducing freshwater toxicity while maintaining weed control, profits, and productivity: effects of increased crop rotation diversity and reduced herbicide usage. Environ. Sci. Technol. 51, 1707–1717.

Inderjit, 2005. Soil microorganisms: an important determinant of allelopathic activity. Plant Soil 274, 227–236.

Inderjit, Foy, C.L., 1999. Nature of the interference potential of mugwort (Artemisia vulgaris). Weed Technol. 13, 176–182.

Jilani, G., Mahmood, S., Chaudhry, A.N., et al., 2008. Allelochemicals: sources, toxicity and microbial transformation in soil- a review. Ann Microbiol 58 (3), 351–357.

Kong, C.H., Wang, P., Gu, Y., et al., 2008. Fate and impact on microorganisms of rice allelochemicals in paddy soil. J. Agric. Food Chem. 56, 5043–5049.

Levin, D., 1971. Plant phenolics-an ecological perspective. Am. Nat. 105, 157–181.

Li, Y.P., Feng, Y.L., Chen, Y.J., et al., 2015. Soil microbes alleviate allelopathy of invasive plants. Sci. Bull. 60, 1083–1091.

Liebman, M., Sundberg, D.N., 2006. Seed mass affects the susceptibility of weed and crop species to phytotoxins extracted from red clover shoots. Weed Sci. 54, 340–345.

Lou, Y., Adam, S.D., Anthony, C., et al., 2016. Interactions between allelochemicals and the microbial community affect weed suppression following cover crop residue incorporation into soil. Plant Soil 399, 357–371.

Ojija, F., Arnold, S.E.J., Treydte, A.C., 2019. Bio-herbicide potential of naturalised *Desmodium uncinatum* crude leaf extract against the invasive plant species *Parthenium hysterophorus*. Biol. Invasions 21 (12), 3641–3653.

Orhan, O., Bulent, I., Gulay, O., 2010. Pretreatment of poultry litter improves *Bacillus thuringiensis*-based bioherbcides production. Bioresour. Technol. 101, 2401–2404.

Pergo, E.M., Abrahim, D., Da, S.C.P.S., et al., 2008. *Bidens pilosa* L. exhibits high sensitivity to coumarin in comparison with three other weed species. J. Chem. Ecol. 34, 499–507.

Schmidt, S.K., 1988. Degradation of juglone by soil bacteria. J. Chem. Ecol. 14, 1561–1571.

Sutherland, J.B., Crawford, D.L., Pometto, A.L., 1983. Metabolism of cinnamic, p-coumaric and ferulic acids by *Streptomyces serotonin*. Can. J. Microbiol. 29, 1253–1257.

Xu, C.C., Ge, Z.W., Li, C., et al., 2019. Inhibition of harmful algae *Phaeocystis globosa* and *Prorocentrum donghaiense* by extracts of coastal invasive plant *Spartina alterniflora*. Sci. Total Environ. 696. UNSP 133930.

Xiao, Z.X., Chang, L., Xu, Z.H., et al., 2017. Vertical leaching of allelochemicals affecting their bioactivity and the microbial community of soil. J. Agric. Food Chem. 65, 7847–7853.

Xiao, Z.X., Lu, S.G., Xu, Z.H., 2019. Biochemistry of allelopathic plant residues in soil. Ekoloji 28 (107), 2997–3006.