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Original article

The impact of cover crop shoot decomposition on soil microorganisms in an apple orchard in northeast China

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ABSTRACT

Mowing can facilitate the incorporation of cover crop shoots into soil and improve the properties of soils in apple orchards. This article evaluated how apple orchard soil responds to the decomposition of the shoot residues of three cover crops[native mixed herbs (NMS), red clover (RCS), and ryegrass (RES)] in terms of microbial metabolism and biomass, and discussed the relationships between microbial responses and shoot chemistry. The chemical composition of shoots was analysed and a buried bag experiment was carried out to simulate shoot decomposition in an apple orchard. The results revealed significant differences in the chemical compositions and shoot C:N ratios (NMS: 10.9, RCS: 19.1, and RES: 12.9) of the three cover crops. The decomposition of the cover crop shoots promoted microbial metabolism and boosted soil bacterial reproduction (increase in the biomass indicator muramic acid: 19.44, 124.15, and 14.83 mg kg^{-1}, respectively. But there are different types of effects on soil fungal reproduction (change in the biomass indicator glucosamine: 712.51, 887.45, and 103.97 mg kg^{-1}), and they are obviously negative, significantly positive, and non-significant respectively. Thus, the native mixed herbs and red clover are preferable swards for better shoot enhancement in apple orchard.

1. Introduction

There have been several measurements of microbial community to assess soil quality, including DNA, phospholipid fatty acid (PLFA) and amino sugar analyses, coupled with community-level physiological profiles (CLPP), which can yield sufficient data to facilitate further management decisions (Grayston et al., 2004; Liang et al., 2008). With regards to assessing and monitoring soil quality (Winding et al., 2005), suggested that a better comprehension of the diversity and functions of microbial communities can be obtained only through continuous research and application of the different technologies that can visibly characterize microbial community in soil. It is a key process that energy and nutrient flow into the terrestrial ecosystems that decompose plant litter. And it is thus decisive to identify the structure and activity of the soil microbial community that plays a significant role in nutrient cycling (Bardgett et al., 2005). The chemical composition of litters is the least heterotrophic part of the complex soil food web, and it can regulate the microbial community structure and richness (Hättenschwiler and Jørgensen, 2010; Breulmann et al., 2012). The changes of substrate quality and quantity and that of environmental conditions can affect Microbial biomass and activity (Vance and Nadkarni, 1990), therefore it is necessary to identify the relationships between litter chemical composition and soil microbial community structure and activity (Bardgett et al., 2005); Numerous studies focus on analysing the correlations between litter chemical composition (e.g. N concentration, lignin: N ratio, C:N ratio and phenolic compound: N ratio), and soil microbial community DNA, PLFAs, amino sugars and CLPP (Meier and Bowman, 2010).

In apple orchard sward ecosystems, herb shoot material is incorporated into the soil after mowing, increasing soil organic matter content. The effects of planting herbs in orchards on soil properties have been extensively studied (Monteiro and Lopes, 2007; Gómez et al., 2009). The organic matter content of orchard soil in China is generally low. In order to increase the content of organic matter in the orchard soil, fruit tree research institutes

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are vigorously promoting the replacement of cover crops to traditional clean tillage in orchard soil management. In apple orchard sward ecosystems, herb shoots are incorporated into the soil via mowing and wilting, Therefore, The utilization rate of soil organic matter content is improved. Although the influence of planting herbs on orchard soil properties has been extensively studied (Monteiro and Lopes, 2007; Gómez et al., 2009), only a few studies have examined the changes in soil microbiological properties in response to herb shoot input. Generally, the effects of roots and leaves on the soil are studied at the same time. In apple orchard sward ecosystems, the time and quantity of herb shoot input can be manipulated by mowing, and thus studying the effects of decomposition on soil individually can have practical value. In the present study, a buried bag experiment was carried out to simulate decomposition of the shoots of three cover crops in the apple orchard. We observed and recorded the the response of soil microbial carbon metabolism and biomass and discuss the relationships between the observed microbial responses and herb shoot chemistry. The main purpose of this research was to appraise the effect of the decomposition of the shoots of three cover crops on the soil microbial metabolism and biomass in an apple orchard.

2. Materials and methods

2.1. Herb shoot collection

Experiments were conducted in an apple orchard in Shenyang Agricultural University (41°83′N, 123°56′E), which has been an experimental interplanting site for a long time. The soil type of the orchard is an acrisol (pH, 6.3; soil organic matter, 8.6 g kg^{-1}; available N, 50.6 mg kg^{-1}; available phosphorus 35.2 mg kg^{-1}; available K, 77.0 mg kg^{-1}). Apple trees ('Hanfu'/GM256/*Malus baccata* Borkh) were planted at a 2.0 m (within-row) × 4.0 m (between-row) density, and different herbs were grown between the tree rows in the spring of 2009. Three types of forage crop (mixed herbs, a legume, and a grass) that showed the best growth condition were selected for this research: native mixed herbs [dominant species included *Echinochloa crusgalli* (L.)Beauv., *Digitaria sanguinalis* (L.) Scop., *Amaranthus viridis* L., *Portulaca oleracea* L., *Amaranthus retroflexus* L., *Chenopodium album* L., *Polygonum caespitosum* Bl., *Acalypha australis* L., and *commune (Commelina Communis L.)*], red clover (*Trifolium pratense* L.), and ryegrass (*Lolium perenne* L.).

In 2012, herb shoot residues of the three cover crops from 25 cm × 30 cm quadrats (three replicates for each) were collected on 27 June, 29 July, and 3 September. The herb shoot residues were air dried, then mixed to a single composite. One portion of the shoots residues samples were grounded into fine powder and was used for chemical analyses, and another was cut to 2-cm pieces and used in the buried bag experiment.

2.2. Analyses of herb shoot chemical composition

The chemical constituents were characterized by pyrolysis-gas chromatography-mass spectrometry (py-GC/MS) (Buurman et al., 2005). Usually, 100 μg of pulverised litter was pulse-pyrolysedin quartz tubes in a Pyroprobe 5150 pyrolyser (CDS Analytical Inc., Oxford, PA) at 590 °C for 10 s. Pyrolysed samples were transferred to the gas chromatograph (7890B/5977A GCMS, Agilent, USA) with He as the carrier gas (flow rate = 1.0 mL·min^{-1}). Peaks were identified by using the mass spectral library of National Institute of Standards and Technology (NIST) and the Automated Mass Spectral Deconvolution and Identification System (AMDIS V 2.65) (Grandy et al., 2008). Relative compound abundances were calculated by normalising the peak intensity of each compound to the sum of the peak intensities for the sample. We grouped pyrolysis products by their source and divided them into the following categories: polysaccharides, lignins, lipids, nitrogenous compounds, and unidentifiable compounds. The latter were categorised as 'unknown' (Wickland and Neff, 2008). Total C and N were measured from three replicates per sample using a CE-440 Elemental Analyser (Exeter Analytical Inc.).

2.3. Herb shoot decomposition in the apple orchard

Initially, samples of the three cover crop shoot residues were dried at 105 °C for 10 h to facilitate dry heat sterilisation. Thereafter, three pieces of fresh cover crop apple orchard soils (collected from a depth of 0–10 cm) were sieved by using a 0.425-mm sieve to remove impurities. In each experimental unit, there were buried bags in which the ratio of fresh soils to dry herb shoot biomass is 100:1 (Breulmann et al., 2012) (200 g soil mixed with 2 g shoot) and bags that contained only 200-g soil (control). The buried bags used for the three experimental units contained the following elements: NMS soil: 200 g native mixed herb sward soil from the apple orchard mixed with 2 g natural mixed herb shoots; no-NMS soil: 200 g native mixed herb sward from the apple orchard soil alone; RCS soil: 200 g red clover sward apple from the apple orchard mixed with 2 g red clover shoots; no-RCS soil: 200 g red clover sward soil from the apple orchard alone; RES soil: 200 g ryegrass sward soil from the apple orchard mixed with 2 g ryegrass shoots; and no-RES soil: 200 g ryegrass sward soil from the apple orchard alone. The soils were wetted with sterile water until the moisture rate reached 60%, which is the maximum field moisture rate (Breulmann et al., 2012), and then the soils were transferred to tightly-bound nylon mesh bags (8 cm × 10 cm) whose pore size is at 0.18 mm. Each unit was comprised with 15 treatments and 15 control bags. Accordingly, there were totally 90 bags for the experiment.

The bags were buried in an apple orchard to simulate the decomposition of shoot residues under field conditions. On 17 May 2013, five 50-cm × 50-cm quadrat plots for each type of apple orchard sward (five repeats for each treatment, each repeat contains three bags of soil mixed with herb shoot and three bags of soil alone as the control group) were randomly selected. Totally there were 15 plots for the three cover crops. In order to eliminate the influence of herb root systems, all the herbs in each plot were pulled out. In each plot, three treatment bags and three control bags were buried, and an approximately 2-cm-thick layer of soil was covered upon them. The plots were managed with clean tillage.

During the 10-week experimental decomposition process, the precipitation and the average temperature of this area were 364.7 mm and18.1 °C, respectively. Heavy rainfall and high temperatures can promote decomposition. The bags from one plot of each herb type were collected at 2-week intervals (i.e. at 2, 4, 6, 8, and 10 weeks). Soils from the triplicate bags were mixed and sieved to remove residual shoots by using a 0.425-mm sieve. These soils were used for subsequent analyses.

2.4. Soil microbial community-level physiological profiles

The soil microbial CLPPs were determined with a Biolog-Eco Plate™ Fresh soil, which is equivalent to adding 10-g fresh soil to a 150-mL flask that contains 90 mL of sterile 0.85%NaCl solution for each soil sample, and the flask is waggled at 200 rpm for 30 min, You can sit at room temperature for 40 min. Ten millilitres of the supernatant were transferred to a 150-mL flask with a sterile pipette and the supernatant was serially diluted to 1:1000 with sterile 0.85% of NaCl solution as vaccination. Suspensions were inoculated in 150-μL wells of a Biolog ecological microporous plate

(Biolog, Hayward, CA, USA) and continuously cultured for 168 h in a camera obscura at 25 °C. Finally, Enzyme-linked immunosorbent assay (ELISA) enzyme labeling (Biolog, Hayward, CA, USA) was used to read the optical density at 590 nm every 24 h. The optical density measurements were used to figure average well colour development (AWCD) in response to microbial metabolic activity in soil, which is an important target of soil microbial usage of a single carbon source, and was assessed via principal component analysis (PCA).

2.5. Analysis of amino sugars

Soil from the buried bags was air-dried and grounded (<0.25 mm) after 10 weeks. Glucosamine (GlcN) and muramic acid (MurN) were detected via the method of Zhang and Amelung (1996). In short, after hydrolysis with 6 mol·L^{-1} HCl for 8 h, the solution was filtered and the pH was adjusted to 6.6–6.8 via centrifugation and freeze-drying. We should wash amino sugars that are used to Methanol from the residues. The amino sugars were transformed into aldononitrile derivatives and then went through a second extraction in methylene dichloride. Excess anhydride was eliminated by 1 mol·L^{-1} HCl and water. After removing dichloromethane with a N_2 stream, the amino sugar derivatives were redissolved in a mixture of hexane and ethyl acetate (1:1 v/v) for final analysis. Myo-inositol was added as an internal standard before hydrolysis, and methyl-glucamine was added as the recovery standard before derivatisation. The amino sugar derivative is classified as an Agilent 7890A (Agilent, USA) gas chromatograph equipped with a HP-5 fused silica column (30 m × 0.25 mm; 0.25 μm). The temperature programme and device parameters were set according to (He et al., 2006).

2.6. Data analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used to process data. Differences between herb shoot residues and buried bag soils were analysed using a one-way ANOVA, And the TUKY (LSD) test has the least difference (P ^ 0.05). CLPPs were procured using multivariate PCA (Wang et al., 2007)

3. Results

3.1. Herb shoot chemistry

There were significant differences in the total C and N contents of the three herb shoot types (Table 1, $P < 0.05$) and the rank is in the following order: total carbon content, RCS > RES > NMS; total nitrogen content, NMS > RES > RCS; and C:N ratio, RCS > RES > NMS.

On the basis of PY-GCMS analysis, we identified 95 compounds in the shoots of the three cover crops, which originated from 43, 20, 7, 20, and 5 probable polysaccharides, lignins, lipids, nitrogenous compound and unknown sources, respectively. A comparison of the three cover crops indicated that NMS had a higher relative abundance of polysaccharides, but a lower amount of lipids. RCS had a higher relative abundance of lignins, lipids, and N-containing compounds, but a lower amount of polysaccharides and unknown compounds, and RES had a higher relative abundance of unknown compounds, but a lower amount of N-containing compounds (Fig. 1).

Table 1
Total carbon and nitrogen contents in herb shoots.

Herb shoot type	C (mgkg^{-1})	N (mgkg^{-1})	C:N ratio
NMS	*385.5 ± 0.8 c*	*35.3 ± 0.9 a*	*10.9 ± 0.3 c*
RCS	*408.8 ± 0.7 a*	*21.5 ± 0.5 c*	*19.1 ± 0.4 a*
RES	*397.4 ± 0.7 b*	*30.9 ± 0.6 b*	*12.9 ± 0.2 b*

Values are the mean ± SE. Values in the same column followed by different letters are significantly different ($P < 0.05$), n = 3. NMS: native mixed herb shoots, RCS: red clover shoots, RES: ryegrass shoots.

3.2. Effect of herbs shoot decomposition on soil microbial CLPP

Dynamic curves of the temporal change in AWCD values indicated that shoot decomposition in all the three cover crops led to a considerable increase in apple orchard soil microbial metabolic activities for five sampling times (Fig. 2). Comparisons among shoots of the three types of herb indicated that the decomposition of native herb shoot residues had the greatest effect on improving the carbon metabolism of soil microorganisms, whereas ryegrass shoot decomposition clearly had the least positive effect compared with that of the other two cover crops. This difference can be attributed to the fact that decomposition of mixed-type plant litter is more efficient than that of a single litter type (Hättenschwiler and Jørgensen, 2010). The amounts of carbon and nitrogenous compounds produced by mixed-litter decomposition were 2.7 and 1.4-times higher than those produced by single-litter decomposition, respectively (Szanser et al., 2011). Therefore, we inferred that higher increase in soil microbial carbon metabolism promoted by the decomposition of native mixed herb shoot residues (Fig. 2) is ascribed to the mixed-litter decomposition effect.

Shoot decomposition of the three cover crops affected the microbial carbon substrate utilisation profiles in apple orchard soil at all five sampling times (Fig. 3). The effects of native herb and red clover shoot decomposition were significant, whereas ryegrass shoot decomposition clearly had a weaker effect in this regard.

3.3. Effects of the decomposition of shoot residues on soil bacterial and fungal biomass

MurN, a unique bacterial compound, is a component of the peptidoglycan in bacterial cell walls (Bird and McClure, 1976; Amelung et al., 2001). Thus, the content of MurN can reflect the reproductive rate of bacteria (Hongbo et al., 2010). The shoot decomposition of all the three cover crops significantly promoted bacterial reproduction in apple orchard soil compared with the respective control groups (Table 2). Fungi play an important role in garbage decomposition, and bacteria are important only after the leaves are partially decomposed (Pascoal and Cássio, 2004).

Glucosamine (GlcN) is an integral part of the cell wall of fungi, and its content in soil can indicate fungal biomass (Glaser et al., 2004). As GlcN is a stable compound, it readily accumulates to form stable soil organic matter, Therefore, the growth of fungi can increase the GlcN content of soil organic matter (He et al., 2011). Comparisons with the control groups showed that decomposition of the shoots of native herbs, red clover, and ryegrass had three different effects on fungal reproduction, namely significantly negative, significantly positive, and non-significant. Comparisons among the three cover crops indicated significant differences in the content of fungal biomass in soils containing their decomposed shoots (RCS soil > RES soil > NMS soil). The biomass of fungi and bacteria in soil were compared and the decomposition activities of these microorganisms were evaluated. The mass ratio of GlcN/MurN is commonly used to index the relative contribution of bacteria and fungi to soil organic matter turnover (Amelung et al., 2001). Shoot decomposition significantly reduced the mass ratios of GlcN/MurN in all three cover crops, indicating that shoot decomposition makes a larger contribution to bacterial reproduction than to fungal reproduction. Moreover, the GlcN/MurN ratio of ryegrass shoot-amended soil was significantly higher than that of soil amended with shoots of the other cover crops. No

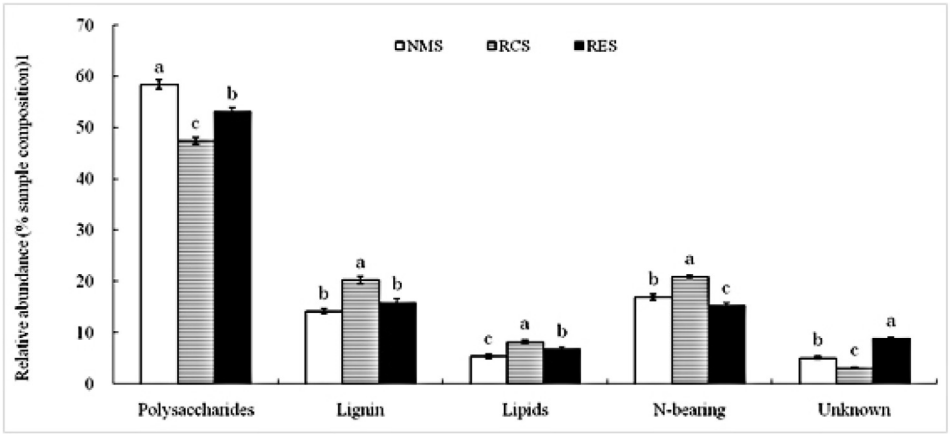

Fig. 1. Relative abundance of chemical source classes in herb shoot residues determined by pyrolysis-gas chromatography-mass spectrometry analyses.

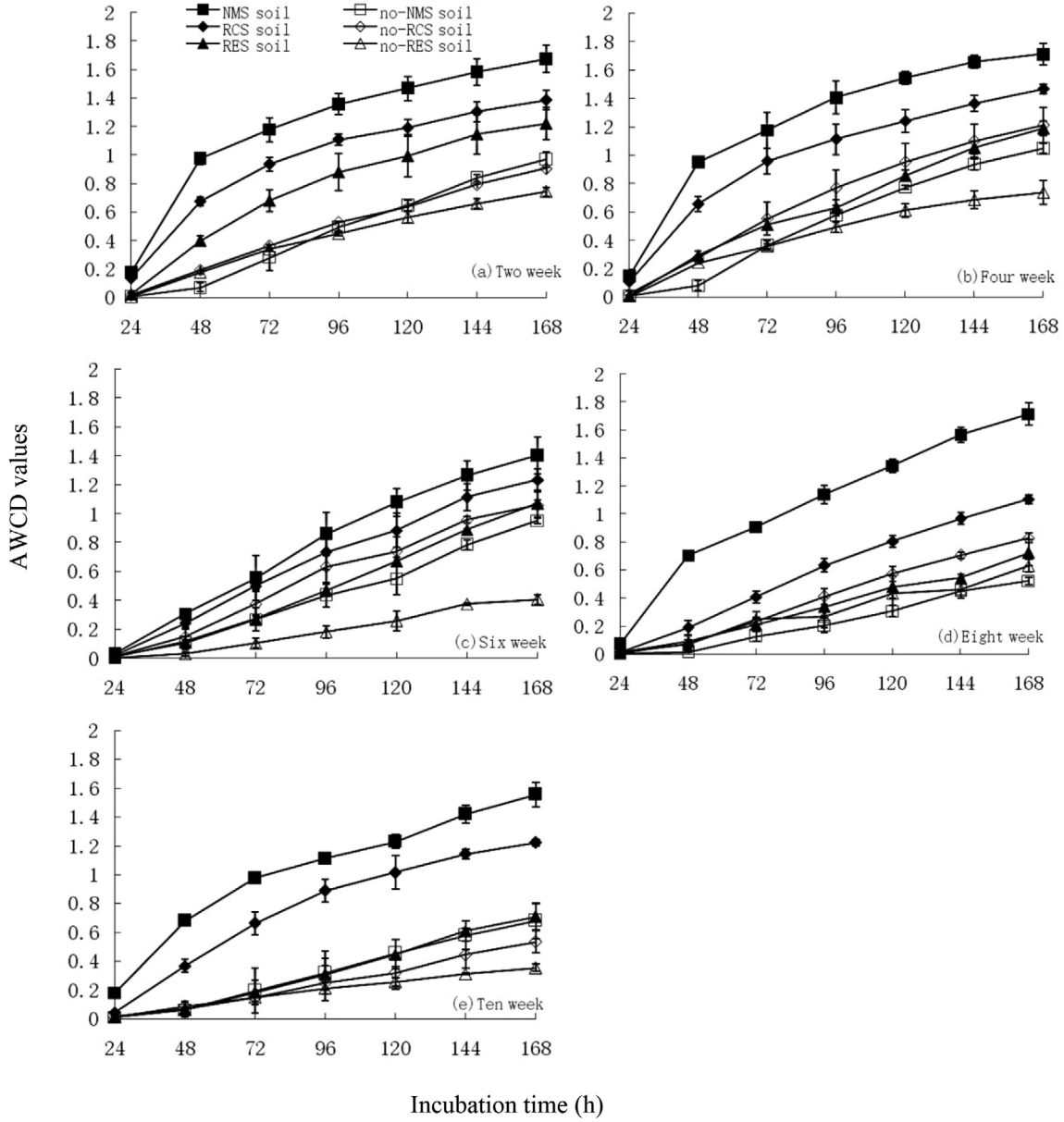

Fig. 2. Dynamic changes in the average well colour development (AWCD) values of buried bag soils at week 2, 4, 6, 8, and 10.

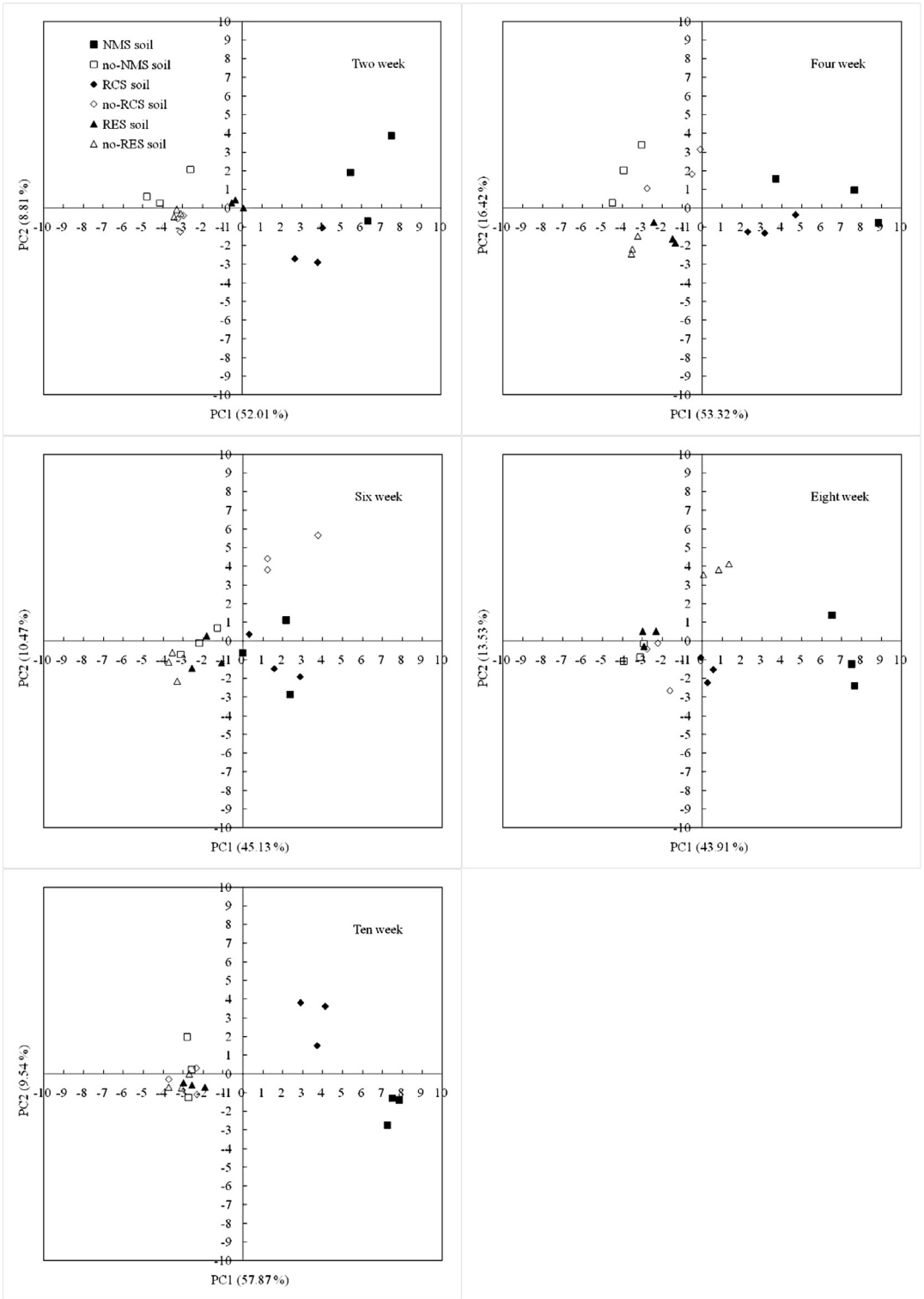

Fig. 3. Principal component analysis of microbial carbon source utilization for buried bag soils at week 2, 4, 6, 8, and 10. PC1: principal component 1, PC2: principal component 2.

significant difference between native herb- and red clover-amended soils was found.

The increase of fungal and bacterial biomass was related to C:N ratio that is associated with the decomposition of shoot residues. A high C:N ratio in the litter is conducive to fungal proliferation, whereas a low C:N value is conducive to bacterial proliferation, and this finding is consistent with the significant positive correlation between fungal proliferation and C:N ratio observed in the present study (Tables 1 and 2). Fungi play an important role in the process of lignin decomposition (Qi et al., 2004), and more than approximately half of the 8500 species of saprophytic fungi reported are able to decompose lignin (Lynch and Thorn, 2006). Accordingly, the higher lignin content of red clover shoots promoted the proliferation of soil fungi (Fig. 1). Some research have

Table 2
Muramic acid and glucosaminein content in soils of buried bag after 10-week.

Soil sample	MurN ($mgkg^{-1}$)	GlcN ($mgkg^{-1}$)	GlcN/MurN ratio
NMS soil	*118.89 ± 7.19 a*	*1323.59 ± 24.04 b*	*11.1 b*
no-NMS soil	*99.45 ± 9.60 b*	*2036.10 ± 97.44 a*	*20.5 a*
RCS soil	*210.20 ± 5.36 a*	*2413.45 ± 54.77 a*	*11.5 b*
no-RCS soil	*86.05 ± 5.31 b*	*1526.00 ± 79.24b*	*17.7 a*
RES soil	*98.96 ± 5.04 a*	*1796.15 ± 93.14 a*	*18.2 b*
no-RES soil	*84.13 ± 2.93 b*	*1692.18 ± 58.23 a*	*20.1 a*
NMS soil	*118.89 ± 7.19 b*	*1323.59 ± 24.04 c*	*11.1 b*
RCS soil	*210.20 ± 5.36 a*	*2413.45 ± 54.77 a*	*11.5 b*
RES soil	*98.96 ± 5.04 b*	*1796.15 ± 93.14 b*	*18.2 a*

Values are the mean ± SE. Values in the same column followed by different letters are significantly different ($P < 0.05$); n = 3. NMS: native mixed herb shoots, RCS: red clover shoots, RES: ryegrass shoots.

found that phenolic derivatives and lignin can, through chemical protection against microbial attack, bypass the microbial carbon pool and direct form steady soil organic matter (Parton et al., 1987). In the present study, fewer native mixed herb shoot and ryegrass shoot residues were visible than red clover shoot residues in the buried bags after 10 weeks, and the latter can promote more stable and larger polymer formation (Ekschmitt et al., 2005). Planting red clover can thus increase the content of soil organic matter because red clover has high C:N ratio (Hättenschwiler and Jørgensen, 2010)and higher lignin content (Osono et al., 2009).

4. Discussion

In this study, we demonstrated that the decomposition of native mixed herb shoots clearly had a greater positive effect on microbial metabolic activities in orchard soil than those of the other two herbs (Fig. 2). This difference can be attributed to the fact that decomposition of mixed-type plant litter is more efficient than that of single-type litter (Hättenschwiler and Jørgensen, 2010). The amounts of carbon and nitrogen compounds produced by mixed-litter decomposition are 2.7 times and 1.4 times, respectively, that produced by single-litter decomposition (Szanser et al., 2011). Therefore, we inferred that the greater increase in soil microbial carbon metabolism (Fig. 2) promoted by the decomposition of native mixed herb shoots (a multispecies shoot mixture) is ascribed to the mixed-litter decomposition effect.

Whereas fungi play a prominent role in the litter decomposition process, bacteria are considered to be of major importance only after leaf material has been partially broken down (Pascoal and Cássio, 2004). In this study, decomposition of native mixed herb, red clover, and ryegrass shoots had three different effects on fungal reproduction, namely significantly negative, significantly positive, and neutral. (Table 2). Glucosamine, a main component of the fungal cell wall, is stable, and it readily accumulates to form stable soil organic matter, thus fungal proliferation can increase the content of soil organic matter (He et al., 2006). Increases in fungal and bacterial biomass are related to the C:N ratio associated with herb shoot decomposition. A high C:N ratio litter is conducive to fungal proliferation, whereas, a low C:N value litter is conducive to bacterial proliferation. The results of the present study are consistent with finding that the proliferation of soil fungi being negatively related to the C:N ratio of herb shoot litter (Tables 1 and 2). Fungi play an important role in the process of lignin decomposition (Qi et al., 2004); and more than approximately half of the 8500 reported species of saprophytic Cignin (Lynch and Thorn, 2006). Accordingly, the higher lignin content of red clover shoots promoted the proliferation of soil fungi (Fig. 1). Some studies have found that lignin and phenolic derivatives can, through chemical protection against microbial attack, bypass the microbial carbon pool and directly form stable soil organic matter (Parton et al., 1987). In the present study, compared with native mixed herb shoot and ryegrass shoot residues, more visible red clover shoot residue was identified in 10-week sampled buried bags, and can promote more highly stable and large polymer formation (Ekschmitt et al., 2005). Planting red clover can thus increase the content of soil organic matter (as observed in a previous long-term investigation), because red clover has high C:N ratio and higher lignin content.

5. Conclusion

Overall, compared with ryegrass, the shoots of native mixed herbs and red clover had superior chemical composition and their decomposition had a more positive influence on apple orchard soil microbial communities. Accordingly, native mixed herbs or red clover would be preferable to ryegrass as an option for apple orchard swards. Furthermore, many previous studies have reported conflicting results regarding the relationship between microbial biomass and microbial functions. These studies have shown positive correlations (Horwath et al., 1996; Meyer et al., 1998; Loreau, 2001); negative correlations (Degens, 1998) in various ways; or no correlation (Griffiths et al., 2000; Wardle et al., 2003; Niklaus and Tate, 2006). In the present study, we found that there were no positive or negative correlations between the changes in soil microorganism (bacterial and fungal) biomass (Table 2) and microbial metabolic activities (Figs. 1 and 2). Our results tend to support the opinion of some authors (Griffiths et al., 2000; Wardle et al., 2003; Niklaus and Tate, 2006), which isthat 'the relationship between soil microbial biomass and carbon metabolism is not relevant'.

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