Applied Soil Ecology 119 (2017) 339–344

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

Effects of snow cover plus straw mulching on microorganisms in paddy soil during winter

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ARTICLE INFO

Keywords:
Microorganisms
Phospholipid fatty acid
Winter
Snow cover
Straw mulching

ABSTRACT

Soil microorganisms play key roles in the biogeochemical cycling of soil carbon and are sensitive to changes in the soil microclimate. A field experiment was conducted to investigate the effects of snow cover and straw mulching on microbial communities in paddy soils during winter, and four treatments were established: (i) snow removal with no straw mulching (Sn-SM-), (ii) snow cover with no straw mulching (SC), (iii) snow removal with straw mulching (SM), and (iv) snow cover with straw mulching (SC + SM +). The SC, SM, and, especially, SC + SM + groups had more bacterial, gram-positive (G^+) bacterial, gram-negative (G^-) bacterial, fungal, and total phospholipid fatty acids (PLFAs) than the Sn-SM- group from the freezing stage to the hard frost stage. The ratio of fungal/bacterial PLFAs first decreased, with the highest value in the SC + SM + treatment, from the freezing to the hard frost stages and increased during the thawing stage, while the ratio of G^+/G^- PLFAs showed the opposite trend. The Sn-SM- treatment resulted in a rapid increase in microorganisms during the thawing stage. Our results demonstrated that the SC, SM, and, especially, SC + SM + treatments reduced the losses of microbial biomass from the unfrozen stage to the hard frost stage and prevented an increase in microbial activity during the thawing stage in a paddy system.

1. Introduction

Soil microorganisms play key roles in the decomposition of organic matter and the biogeochemical cycling of soil nutrients in ecosystems (Leininger et al., 2006; Cusack et al., 2011). Soil microorganisms are sensitive to the soil microclimate, such as temperature, so the freeze–thaw action caused by temperature shifts is an important factor that may affect soil biological properties (Yanai et al., 2004; Groffman et al., 2011), such as microbial communities (Sharma et al., 2006), but there is little consensus as to the degree of its influence on soil microorganisms. For instance, some studies have shown a decrease in microbial biomass or cell numbers after thawing (Pesaro et al., 2003), while other reports indicate that freeze–thaw cycles have no effect on microbial biomass (Grogan et al., 2004; Sharma et al., 2006) and that microbial activity is enhanced upon thawing (Sharma et al., 2006; Koponen and Bååth, 2016). Several studies have shown that freezing/hard frost affects soil microbial activity and microbial community composition (Sulkava and Huhta, 2003; Henry, 2007), but others have shown that extreme freezing/hard frost has no effect on bacterial biomass but reduces fungal biomass (Yergeau and Kowalchuk, 2008). Therefore, further research into the effects of freeze-thaw cycles and hard frost on soil microorganisms is necessary.

Snow cover and its duration strongly influence soil temperature and moisture (Rixen et al., 2004), which may be the most important factors affecting microbial activity during winter (Walker et al., 1999). Snow cover may decouple soil temperature from air temperature, preventing the physical changes related to soil freezing and thawing (Steinweg et al., 2008), and snow removal may increase daily variations in soil temperature, alter the frequency of freeze-thaw cycles and affect soil frost depth as well as advance the dates of soil freezing and melting (Tan et al., 2014), which in turn affect soil biochemical processes (Groffman et al., 2011) and soil microbial activity (Tan et al., 2014).

Crop straw mulching is a common cultural practice used to supplement soil organic carbon (Wang et al., 2015), and recent studies have focused on the effects of straw mulching on soil organic carbon (Wang et al., 2015), enzymatic activities (Li et al., 2016; Zhao et al., 2016) and microbial properties (Li et al., 2016; Zhao et al., 2016). Straw mulching increased soil organic carbon levels (Zhu et al., 2015; Zhao et al., 2016), water-soluble organic carbon (Li et al., 2016) and labile organic carbon fractions (Zhu et al., 2015) as well as stimulated

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http://dx.doi.org/10.1016/j.apsoil.2017.05.023
Received 10 March 2017; Received in revised form 17 May 2017; Accepted 24 May 2017
Available online 23 July 2017
0929-1393/ © 2017 Published by Elsevier B.V.

enzymatic activity and bacterial counts (Lou et al., 2011), and distinct shifts in soil bacterial and fungal community compositions (Yang et al., 2016) were observed. In contrast, Zhao et al. (2016) found that straw mulching might or might not have significant effects on microbial community structure. However, because these studies focused on the growing season, little is currently known about how straw mulching combined with snow cover affects microbial communities in paddy soils during winter in a seasonally frost-affected region.

Western Jilin Province is a major grain production region in China, and paddy fields represent one of the main land-use types. The study area has a typical arid continental monsoonal climate that is very cold in winter with a large amount of snowfall and very dry in spring with quickly rising temperatures accompanied by limited rainfall. Freeze-thaw events occur in early winter and early spring, between which the soil is frozen completely and experiences hard frost. However, until now, little has been known about the dynamics of microbial communities under seasonal snow cover in this region, so the overall objectives of this study were to (1) assess the impacts of snow cover and rice straw mulching on microbial communities and (2) reveal variations in microbial communities during winter under natural conditions.

2. Materials and methods

2.1. Study area and site selection

Field experiments were carried out from October 2013 to April 2014 in a single field in Songyuan City (123°6′–126°6′N, 436°6′–456°6′E) in Western Jilin Province, China. The experimental soil was paddy soil (chernozem) with a pH of 7.8 that contained 25.3 g/kg organic carbon, 145 mg/kg available N, 48 mg/kg available P and 135 mg/kg available K with 34.6% moisture in the 0–15-cm layer in the unfrozen stage.

2.2. Experimental design and treatments

The experiment consisted of four treatments: (i) snow removal with no straw mulching (Sn-SM-); (ii) snow cover with no straw mulching (SC); (iii) snow removal with straw mulching (SM), and (iv) snow cover with straw mulching (SC + SM +), which were arranged in a randomized complete block design with three replicates. After the rice was harvested in October, 2013, dry rice straw was uniformly and completely spread on the soil surface to a thickness of approximately 5.0 cm for all straw treatment groups. Each plot measured 4 m^2 (2 m × 2 m) and was insulated by three layers of heat-insulated plastic board (40 cm × 200 cm) buried to a depth of 40 cm below the soil surface to minimize the movement of microbes between plots via lateral water movement.

2.3. Soil sampling

Samples were collected on 28th October, 2013 (unfrozen stage); 5th December, 2013 (freezing stage: when the 0–15-cm soil layer of all groups was completely frozen); 10th February, 2014 (hard frost stage: when the soil in all groups had been frozen for a long time and was under hard frost); and 23rd April, 2014 (thawing stage: when the 0–15-cm soil layer of all groups had completely thawed). Soil samples were collected from 0 to 15 cm from the four treatments using soil drills, which were cleaned with distilled water before any sampling. Three randomly distributed, parallel samples were collected in each treatment.

2.4. Measurements of temperature, frost thickness and snow cover thickness

Measurements of temperature, frost thickness and snow cover thickness were performed from 28th October, 2013 to 23rd April, 2014 at 8:00 am approximately every 3 to 4 days. Frost thickness and snow cover thickness were detected using a frozen soil apparatus (Type: TB1-1, made in China), which was composed of an internal and external pipe with the bottom and top ends sealed. The external pipe was 100 cm long and marked with a scale; 50 cm of the pipe was inserted underground with the remainder aboveground to measure snow cover thickness. The transparent internal pipe was 50 cm long with a scale, contained a chain and clear water and was inserted vertically into the bottom of the external pipe; frost thickness was determined by measuring the height of the frozen water inside the pipe. Soil temperature was measured using an electronic geothermometer (Type: TP101, made in China), which ranged from −50 °C to 300 °C with a testing length of 15 cm.

2.5. Measurements of microbial phospholipid fatty acids PLFAs

Soil microbial community structure was determined by PLFAs analysis according to a modified version of the Bligh–Dyer method (Brant et al., 2006a). Fresh soil (3 g) was used for PLFA extraction with a single-phase mixture of chloroform–methanol–citrate buffer (1:2:0.8 volume ratio), and the extracted lipids were separated by the silica-bonded phase. The PLFAs were trans-esterified to fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. The FAMEs were quantified by Agilent 7890 gas chromatography (Agilent Technologies, Santa Clara, CA, USA) and identified using the MIDI Sherlock system (MIDI Inc., Newark, DE, USA); the 19:0 fatty acid was used as an internal standard. Bacteria were identified by the following PLFAs: 11: 0, 12: 0, 13: 0, 14: 0, α14: 0, i13: 0, i14: 0, 15: 0, i15: 0, α15: 0, 16: 0, i16: 0, i17: 0, 16: 1ω7c, cy17: 0, 17: 0 and cy17: 0., etc. (Brant et al., 2006b; Djukic et al., 2010). Gram-positive bacteria (G^+) were identified by the following PLFAs: i14: 0, i15: 0, α15: 0, i16: 0 and i17: 0 and Gram-negative bacteria (G^-) by 10: 0 2OH, 12: 0 2OH, 14: 0 2OH, 16: 0 2OH, 16: 1ω7c, cy17: 0 and cy19: 0 (Bach et al., 2010). The PLFAs 18: 2ω6c, 18: 1ω9c and 18: 1ω9 t were chosen to represent fungi (Fierer et al., 2003).

2.6. Statistical analyses

Multiple comparisons were conducted using SPSS 22.0 software (International Business Machines Corporation, State of New York, America) and the LSD method. Figures were drawn using SigmaPlot 12.5 (Systat Software, Inc., State of Illinois, America) and Excel 2013 (Microsoft Corporation, State of Washington, America).

3. Results

3.1. Variations in bacterial PLFAs

The concentrations of bacterial PLFAs did not differ significantly from the unfrozen stage to the freezing stage in all treatments (Fig. 1). However, they decreased by 4.3, 6.0, 4.1, and 8.2 nmol/g and reached a minimum value in the SC, SM, SC + SM + and Sn-SM- treatments, respectively, and the Sn-SM- treatment had the least and the SC + SM + treatment had the most bacterial PLFAs at the hard frost stage. During the thawing stage, bacterial PLFAs increased by 4.9, 7.7, 3.7, and 12 nmol/g in the SC, SM, SC + SM + and Sn-SM- treatments, respectively, and the Sn-SM- treatment exhibited the most bacterial PLFAs while the SC + SM + treatment had the least.

3.2. Variations in G^+ and G^- PLFAs

The freeze-thaw process in the early winter did not significantly impact G^+ and G^- PLFAs from the unfrozen stage to freezing stage (Fig. 2), but G^+ PLFAs decreased by 1.0, 1.2, 1.0, and 1.8 nmol/g while G^- PLFAs decreased by 1.3, 1.6, 1.3, and 2.2 nmol/g in the SC, SM, SC + SM + and Sn-SM- treatments, respectively, in the hard frost stage. Both G^+ and G^- PLFAs reached a minimum value in all treatments during hard frost stage, and for both G^+ and G^- PLFAs, the Sn-SM-

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