March 23 2018

A Response to Reviewers

Dear Anja Rammig,

We would like to thank you and the two reviewers for the thoughtful and valuable suggestions on our manuscript entitled "Understory vegetation plays the key role on sustaining soil microbial biomass and extracellular enzyme activities" (bg-2017-545). We have carefully revised our manuscript to take account of your comments and suggestions. Please find below our responses (color-coded blue) to **Editor's** and **Reviewer's** comments (repeated *in an italic font*). The page and line numbers mentioned here refer to the latest revision of our unmarked manuscript.

Comments from Editor

Please upload the revised version of the manuscript.

Response:

We have uploaded the revised version of the manuscript.

Comments from Reviewers

Anonymous Referee #1:

The authors Yang et al., present timely results of microbial abundance and activity in fir planted soil with and without understory removal

Strength of MS:

Hot topic Broad indicator Setup

Weakness of MS:

Hypotheses lack on novelty and a concise discussion

Discussion shows serious flaws such as Content and style of writing, which should be strongly improved

The content of discussion should be improved, as the authors showing really interesting data (Suggestions are attached)

Nevertheless, I think this study is worth for publication in BG after a careful improvement of the MS

1. Please use the right terminology throughout the MS. eg. Content not conc. or organic C not soil C and many more (find in the attached PDF)

Response:

We have revised the inaccurate terms throughout the manuscript according to your comments, such as changed "soil environmental factors" to "soil abiotic properties", changed "soil C (DOC, POC and SOC)" to "soil organic C (DOC, POC and SOC)", and changed "concentration" to "content", and changed "microbial biomass" to "PLFA contents".

2. Hypothesis should be more attractive to the reader (example attached)

Response:

We have revised the Hypothesis as "We hypothesized that the removal of understory vegetation decreased rhizodepostion and therefore microbial biomass and activity." according to your suggestion.

3. Results should be clarified eg. MBC vs. PLFA

Response:

We have revised "microbial biomass" to "PLFA contents" throughout the manuscript.

4. Inferences should be drawn newly and in accordance to the literature eg. NAG is also in bacterial cells or AP activity is higher compared to others does not mean automatically that there is a P Limitation.

Response:

We have revised the sentence "Chitin, a major structural component of fungal cell wall, and peptidoglycan, a major structural component of bacterial cell wall (Loeppmann et al., 2016b), can be degraded by NAG (Mganga et al., 2015)".

"In highly weathered red soil in southern China, P is the most limiting element, and most soil P is presented in organic form or is immobilized by high contents of Al and Fe (Margalef et al., 2017). Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was limiting nutrient in red soil. Soil microorganisms may produce more phosphatase to mineralized organic P and release phosphate to meet their demand for P (Allison and Vitousek, 2005)".

5. Suggest to calculate different Indices to improve your inferences accordingly e.g. Specific Enzyme activity or Enzyme Indices (Moorhead et al., 2013; Loeppmann et al., 2016)

Response:

We have analyzed the specific enzyme activities normalized by total PLFAs, as

well as the stoichiometry of enzyme activity through calculating the ratios of C/N and C/P potential acquisition activity, as indicated by the ratios of ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP, respectively.

6. M & M Section: Should be strongly improved - more details (See my comments attached)

Response:

We have revised the Material and Methods section according to your comments, such as, we have added the soil classification "The main soil in the study area is classified as Udults using the USDA-NRCS soil taxonomy (1996)"; we have explained the buffer zone was set between each plot as "to avoid the influence between each plot"; we have rephrase the plots design as "Each plot was divided into four 15 m × 15 m subplots and contained two treatments: understory vegetation and litter removal (None) and understory vegetation left intact but litter removal (Understory). The two subplots with the same treatment in one plot were distributed across each plot to avoid the effects of slope (Fig. 1) and were averaged as one analysis replication. The litter and understory were managed on a monthly basis. For the **None** treatment, we removed all litter and understory vegetation from the plot. For the **Understory** treatment, we removed the litter from the plot, but left the understory vegetation intact".

We have described the determination method of soil enzymes in more detail as "Soil enzyme activities were measured following the methods of Saiya-Cork et al., (2002). The specific substrates and functions of the enzymes assayed are listed in Table A1. Five hydrolase activities (α-1,4-glucosidase, β-1,4-glucosidase (βG),

β-1,4-N-acetylglucosaminidase (NAG), β-1,4-xylosidase (βX) and acid phosphatase (AP)) were assayed using fluorogenically-labeled substrates. Briefly, a soil suspension was prepared by adding 1 g of fresh soil to 125 mL of 50 mM acetate buffer. We added 200 μL of the soil suspension and 50 μL of the substrate solution (200 μM) to 96 microplates in eight analytical replicates. Methylumbelliferone (MUB) was used for calibration of hydrolase activities. The microplates were incubated in the dark at 20 ℃ for up to 4 h. After incubation, 10μL of 1 M NaOH was added to each well to terminate enzymatic reaction. Following termination of each reaction, the fluorescence was measured using a microplate fluorometer (SynergyH4, BioTek) with excitation and emission filters of 365 nm and 450 nm, respectively".

7. It is not clear why just some data was analyzed with time? Suggest to show all data throughout the whole samplings

Response:

The data we have presented in the text was the average data of April, July and November. And we have also presented the data of soil abiotic and biotic properties, such as PLFA contents, and extracellular enzyme activities in different months in the Supplementary Material (Table A4, A5 and A6).

Comments Attached of Anonymous Referee #1:

1. Line 1 Replace "a" with "the", "in" vielleicht on?

Response:

The title has been revised as "Understory vegetation plays the key role on

sustaining soil microbial biomass and enzyme activities" (Line 1).

2. Line 12 Delete "It is desirable to learn more how".

Response:

"It is desirable to learn more how" was deleted.

3. Line 14-15 Replace "soil properties" with " abiotic and biotic soil properties". "through an examination of the effects of understory vegetation on soil environmental factors" better solely write such as.

Response:

We have revised the sentence "The aim of this study was to determine the role of understory vegetation in controlling soil properties, through an examination of the effects of understory vegetation on soil environmental factors, microbial biomass, and extracellular enzyme activities" to "The aim of this study was to determine the role of understory vegetation in controlling soil abiotic and biotic properties, such as PLFA contents, and extracellular enzyme activities".

4. Line 18 "soil environmental factors" better go with the terms biotic abiotic throughout the MS.

5. Line 19-20 Add "and two oxidative enzymes" after "five hydrolases". "i.e., α-1,4-glucosidase, β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase and acid phosphatase (AP), and two oxidase, i.e., phenol oxidase (PPO) and peroxidase (PER)" not needed in your abstract.

Response:

We have revised the sentence "We mainly evaluated the effects of understory

vegetation on soil environmental factors, the biomass of bacteria, fungi and actinomycetes, and the activities of five hydrolases, i.e., α-1,4-glucosidase, β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase and acid phosphatase (AP), and two oxidase, i.e., phenol oxidase (PPO) and peroxidase (PER)" to "We mainly evaluated the effects of understory vegetation on soil abiotic properties, the PLFA contents of bacteria, fungi and actinobacterias, and the activities of five hydrolases and two oxidative enzymes". And we have revised all "soil environmental factors" to "soil abiotic properties" throughout the manuscript.

6. Line 21 Delete "and the",

7. Line 22 Delete "and". Replace "nitrogen" with "(N)".

8.Line 23 "4% to 34%" values not clear, splitt the sentence. Replace "and" with "as well as"

9. Line 24 Replace"between 13% and" with "up to". "understory vegetation" add "the".

Response:

We have revised the sentence "The soil moisture content (SMC), and the concentrations of soil dissolved organic carbon (DOC), particulate organic carbon (POC), soil organic carbon (SOC), ammonia nitrogen (NH_4^+-N), and total nitrogen, and the POC/SOC ratio declined by 4% to 34%, and the biomass of soil bacteria and fungi, total PLFA contents, and the activities of βG, NAG, PPO, and PER were between 13% and 27% lower, when understory vegetation was removed" to "The soil moisture content (SMC), contents of soil dissolved organic carbon (DOC),

particulate organic carbon (POC), soil organic carbon (SOC), ammonia nitrogen (NH_4^+-N), total nitrogen (TN), and the POC/SOC ratios respectively declined by 4%, 18%, 25%, 12%, 34% and 12%, and soil bacterial, fungal and total PLFA contents, and the activities of β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase (NAG), phenol oxidase (PPO), as well as peroxidase (PER) were up to 27% lower, when the understory vegetation was removed".

10 Line 24-25 "The highest activity of AP among all the measured enzymes may reflect the P was limited in this area" Suggest to delete this sentence, since a higher AP activity compared to other enzyme activities does not lead to the conclusion, that there is a P limitation. Replace "AP" with "acid phosphatase".

11. Line 26 "reflected that P- and N- degrading enzyme affected by different mechanism" common knowledge as these enzymes belong either to N-cycling enzymes or P-cycling enzymes, which both are produced by plants (understory and none-understory) and microbes.

Response:

We have deleted the sentence of "The highest activity of AP among all the measured enzymes may reflect the P was limited in this area, while NAG was positive with the concentration of NO_3^--N, reflected that P- and N- degrading enzyme affected by different mechanism".

12. Line 26 "The positive relationship between DOC and AP implied that microorganisms absorb carbon to meet their needs for phosphorus." this statement is not clear to me. Increased DOC contents may be linked to increased root

exudation which may increase MBC and therefore to increased P acquistion. If that make any sence, though

Response:

We have revised "The positive relationship between DOC and AP implied that microorganisms absorb carbon to meet their needs for phosphorus" to "The positive relationships between DOC and acid phosphatase (AP) implied that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition".

13. Line 30 wording "energy". Rephrase that sentence.

Response:

We have revised "Understory vegetation removal inhibited the propagation of microorganisms and restricted their enzyme activities, by reducing soil energy and above-ground nutrient inputs and altering the soil micro-environment" to "Understory vegetation alter soil microbial biomass, which may influence the decomposition of soil organic matter, by changing soil carbon inputs."

14. Line 38 Replace "soil process" with "soil processes". "Lamb et al., (2011)" correct citation please.

15 Line 39 "under-ground root inputs" Do you mean rhizodeposition? Not clear at all. Delete "the". "forest ecosystem" add "s".

Response:

We have revised "Understory vegetation removal influence soil process by reducing above-ground plant diversity Lamb et al., (2011) and biomass (Fu et al., 2015) and changing under-ground root inputs quality (Li et al., 2013) in the forest

ecosystem" to "Understory vegetation removal influence soil processes by reducing above-ground plant diversity (Lamb et al., 2011) and biomass (Fu et al., 2015) and changing under-ground rhizodeposition quality (Li et al., 2013) in forest ecosystems."

16. Line 40 "moisture" better name it water.

17. Line 40 "it also releases C and nutrients to soils" What kind of C and nutrients are released? What about mucilage?

18. Line 41 Replace "the" with "through".

Response:

We have modified "While understory vegetation absorbs moisture and nutrients from the soil (Wang et al., 2014), it also releases C and nutrients to soils through root exudates, and the turnover of fine roots and leaf litter (Liu et al., 2012)." to "While understory vegetation absorbs water and nutrients from soil (Wang et al., 2014), it also releases carbohydrates, such as sloughed-off root cap and border cells, mucilage and exudates through root (McNear Jr, 2013) and cellulose, hemicelluloses and lignin in the form of leaf litter (Loeppmann et al., 2016a, b), to soils".

19. Line 41-43 "The net effect of understory vegetation on soil nutrients is therefore the balance between the understory vegetation's nutrient demand and its capacity to release nutrients to the soil" I can think of the meaning of the sentence the authors wanted to mention. I suggest to rephrase that sentence.

Response:

We have modified this sentence to "The net effect of understory vegetation on soil

nutrients is decided by the balance between the understory vegetation's nutrient demand and its capacity to release carbohydrates to soil via the decompositions of understory derived litter and rhizodeposition".

20. Line 43-46 "Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Stone et al., 2014), in line with the nutrient requirements of plants and microorganisms to ensure the nutrient balance is maintained the context of the changes in soil environment (Burns et al., 2013)." split this sentence. Why the authors citing Stone throughout the MS? This was found much earlier, methods were developed by others.

Response:

We have changed this sentence to "Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Burns et al., 2013; Nannipieri et al., 2018). Individual enzyme activities reflect the nutrient requirements of microorganisms and the microbial strategies for maintaining the nutrient balance in response to changes in the soil environment (Burns et al., 2013)".

21. Line 52 "respiration" which respiration?

Response:

We have revised "respiration" to "soil respiration".

22. Line 59 Replace "The brief review therefore shows that there is inconsistency in the" with "There is inconsistent".

23. Line 60 delete "with some studies".

24. Line 61 Replace "and" with "or". Delete "others reporting that they".

25. Line 62 Replace "in the" with "under".

Response:

We have revised the sentence of "The brief review therefore shows that there is inconsistency in the information currently available about the responses of soil enzyme activities to understory vegetation, with some studies reporting that soil enzyme activities decreased in the subtropical alpine coniferous forest (Huang et al., 2014), and others reporting that they did not change in the *Pinus sylvestris* var. *mongolica* plantation (Lin et al., 2012), when understory vegetation was removed" to "There is inconsistent information currently available about the responses of soil enzyme activities to understory vegetation, reporting that soil enzyme activities decreased in the subtropical alpine coniferous forest (Huang et al., 2014), or did not change under *Pinus sylvestris* var. *mongolica* plantation (Lin et al., 2012), when understory vegetation was removed".

26. Line 73 Delete "We are not sure" Yet, it is still of high interest.

Response:

We have revised the sentence of "We are not sure how the soil enzyme activities are affected by the understory vegetation removal in Chinese fir plantations" to "It is still of high interest how the soil enzyme activities are affected by the understory vegetation removal in Chinese fir plantations".

27. Line 75 Replace "used" with "established". Delete "in the context of without litter"

28. Line 76 Add "biotic and abiotic factors such as" before "soil enzyme activities, microbial biomass". Delete "soil environmental factors". Replace "in" with "at".

Response:

We have revised the sentence “In this study, we used a long-term field experiment to assess how understory vegetation in the context of without litter influences soil enzyme activities, microbial biomass, and soil environmental factors in Chinese fir plantations” to “In this study, we established a long-term field experiment to assess how understory vegetation influences soil abiotic properties, PLFA contents and enzyme activities at Chinese fir plantations”.

29. Line 77 “the nutrient contents release from short-term storage pools” what do the authors mean? Which pools they address? If soil OC pools addressed better do not call them nutrients. “root exudates” better term below-ground C input.

Response:

We have revised “Earlier studies reported that the nutrient contents release from short-term storage pools, such as root exudates, fine root turnover and leaf litter, decreased when understory vegetation was removed (Liu et al., 2012)” to “Earlier studies reported that the labile C release from below-ground C input decreased when understory vegetation was removed (Liu et al., 2012)”.

30. Line 78-80 “ We therefore hypothesized that soil C and nutrient availability, microbial biomass, and enzyme activities would decline upon removal of the understory vegetation” Adjust to We hypothesized that the removal of understory vegetation, decrease rhizodepostion and therefore microbial biomass and activity.

Response:

We have revised “We therefore hypothesized that soil C and nutrient availability, microbial biomass, and enzyme activities would decline upon removal of the

understory vegetation" to "We hypothesized that the removal of understory vegetation decreased rhizodepostion and therefore microbial biomass and activity".

31. Line 80-81 Delate "Furthermore, we expected that our study would highlight". Replace "the microbial biomass, enzyme activities, and soil environmental factors" with "biotic and abiotic soil factors..... gain new insights on forest nutrition"

Response:

We have revised "Furthermore, we expected that our study would highlight the interactions between the microbial biomass, enzyme activities, and soil environmental factors under different forest understory management practices." to "The interactions between soil abiotic and biotic properties under different forest understory management practices could gain new insights on forest nutrition".

32. Line 88 "red soil" colored soil show Munsell values

Response:

The main soil type in this area is red soil (Munsell values: moisture, 7.5 YR 5/6 and dry, 7.5 YR 6/6).

33. Line 89 " Udults" add name classification system.

Response:

"The main soil type in this area is red soil, which forms from red sandstone and sandy conglomerate and is classified as Udults using the USDA-NRCS soil taxonomy (1996)".

34. Line 95 please write why you used buffer zones.

Response:

"Three 30 m × 30 m plots, with a buffer zone between them exceeding 10 m to

avoid the influence between each plot, were established in the Chinese fir plantation in January 2013".

35. Line 96 "within the three plots" refer to each of the three plots???

Response:

We have revised this sentence to "One paired treatment with three replications was established within each of the three plots".

36. Line 96-101 "Each plot was divided into four 15 × 15 m subplots and contained two treatments, the same treatment were distributed across each plot to avoid the effects of slope (Fig. 1). The two subplots with the same treatment in one plot were averaged as one analysis replication. The treatments comprised understory vegetation and litter removal (None) and understory vegetation left intact but litter removal (Understory). The litter and understory were managed on a monthly basis." Please rephrase it more clearly and improve fig 1 accordingly. So that it is easily understandable by reading it the first time. Suggest to improve the structure.

Response:

We have modified this sentence as "One paired treatment with three replications was established within each of the three plots. Each plot was divided into four 15 m × 15 m subplots and contained two treatments: understory vegetation and litter removal (None) and understory vegetation left intact but litter removal (Understory). The two subplots with the same treatment in one plot were distributed across each plot to avoid the effects of slope (Fig. 1) and were averaged as one analysis replication. The litter and understory were managed on a monthly

basis".

37. Line 106-108 When you sample randomly may it be that some of the sampled soil was closer attached to the roots than soil sampled far away from rhizosphere hotspots? With other words you will end up with a pooled soil (rhizosphere soil and bulk soil).

What does as early as possible mean? Be precise.

Response:

The soil we collected was bulk soil, we have written "Bulk soil samples were collected in…." in the modified version. And we have revised "as early as possible" to "prior to analysis".

38. Line 109 "soil temperature" How often you measured? Day or night?

Response:

The soil temperature was measured three times a year, and was measured when sampling (in April, July and November, respectively). We have revised "Soil temperature (ST) was determined at a depth of 10 cm with a soil thermometer (TP101)." to " Soil temperature (ST) was determined at a depth of 10 cm with a soil thermometer (TP101) when sampling".

39. Line 119-123 Some of your measured biomarkers reflecting the MBC are known to occur also in plant cells (Joergensen & Wichern 2008; Zelles 1997) How to handle that problem? That suggest your MBC is lower than presented here.

Response:

A major disadvantage of PLFA analysis is that none of the PLFA biomarker is fully specific for a certain microbial group. For example, plants may contain high

contents of 18:1w9c and 18:2w6,9 (Joergensen & Wichern 2008; Zelles 1997). This may disturb our results and lead to our total PLFA contents higher than actual value. Kaiser et al. (2010) measured the contents of PLFA biomarkers in beech roots and calculated the possible contribution of root-borne PLFAs to eliminate the plant-derived biomarkers. We didn't measure the contents of PLFA biomarkers in plant roots, but we have minimized the impact of plant by sieving and removing roots. We suggest to measure the contents of PLFA biomarkers in plant roots to eliminate the plant-derived biomarker in the future study. And we have changed all "microbial biomass" to "PLFA contents" throughout the manuscript.

40. Line 124-130 Please describe your methods in more detail. Everybody should be able to repeat. Did you do any calibration? If yes please mention.

Response:

We have revised this paragraph as "Soil enzyme activities were measured following the methods of Saiya-Cork et al., (2002). The specific substrates and functions of the enzymes assayed are listed in Table A1. Five hydrolase activities (α-1,4-glucosidase, β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase (βX) and acid phosphatase (AP)) were assayed using fluorogenically-labeled substrates. Briefly, a soil suspension was prepared by adding 1 g of fresh soil to 125 mL of 50 mM acetate buffer. We added 200 μL of the soil suspension and 50 μL of the substrate solution (200 μM) to 96 microplates in eight analytical replicates. Methylumbelliferone (MUB) was used for calibration of hydrolase activities. The microplates were incubated in the dark at 20 ℃ for up to 4 h. After incubation, 10μL of 1 M NaOH was added to each well to terminate

enzymatic reaction. Following termination of each reaction, the fluorescence was measured using a microplate fluorometer (SynergyH4, BioTek) with excitation and emission filters of 365 nm and 450 nm, respectively".

41. Line 135 Why you measured at 460 nm ? Show any publication doing so, cite it though

Response:

We are so sorry to have made a mistake, and we have revised the sentence "We then moved 250 μL of the supernatant to the microplates and measured the absorbance at 460 nm with a microplate fluorometer" to "We then moved 250 μL of the supernatant to the microplates and measured the absorbance at 450 nm with a microplate fluorometer (DeForest, 2009)".

42. Line 139 Did the authors check for normal distribution?

Response:

All of the data satisfy the normal distribution criteria for parameter analysis was tested by one-sample Kolmogorov-Smirnov test using SPSS 17.0.

43. Line 147 As you said you sampled in time. Data is either missing in some figs or not all the data is measured in time. But make it clear throughout your results.

Response:

The data we used in the text was the average data of April, July and November. N=18, n=3. And we presented the data of soil abiotic and biotic properties, such as PLFA contents, and extracellular enzyme activities in different months in the Supplementary Material (Table A4, A5 and A6).

44. Line 150 Replace "concentration" with "content". check throughout MS

Response:

We have replaced all “concentration” with “content” throughout the manuscript.

45. Line 156 It would be helpful to convert total PLFA content to total microbial biomass content to be able to compare it other studies

Response:

We only used PLFA content in our manuscript, and we have changed all “microbial biomass” to “PLFA contents” throughout the manuscript to make it easily understandable.

46. Line 157 Show results of B:F ratios

Response:

We have shown that “ The ratios of fungi/bacteria did not change because the bacterial and fungal PLFA contents decreased simultaneously when understory vegetation was removed”.

47. Line 162 Be precise which enzymes

Response:

We have revised this sentence to “Understory vegetation significantly affected soil enzyme activities. The potential activities of βG, NAG, PPO, and PER were higher in the treatments with understory vegetation than in the treatment without understory vegetation (Fig. 3a and b) ($P < 0.05$)”.

48. Line 163-164 . rephrase to XXX and xxx reduced by xxx and xxx respectively.

Response:

We have revised this sentence to “When the understory vegetation was removed, the potential activities of βG, NAG, PPO, and PER reduced by 13%, 24%, 21%

and 20%, respectively ($P < 0.05$)".

49. Line 165 Replace "phosphate hydrolase activities" with "acid phosphatases".

Response:

We have revised "phosphate hydrolase activities" to "acid phosphatases".

50. Line 173-174 "The concentration of NO_3^--N was positively correlated with G^+, bacteria, actinomycetes, total PLFAs, and G^+/G^-." How you would explain that? Many studies do not show that correlation with NO3 addition.

Response:

We have delete this sentence since the content of NO_3^--N wasn't affected by understory vegetation management.

51. Line 169-176 In the whole paragraph it is not clear if the correlation is referring to all soils or just to certain treatments.

Response:

We refer to all soils. We have clearly mentioned that "We investigated the relationships among soil abiotic properties and PLFA contents and enzyme activities of all soil using redundancy analysis (RDA, CANOCO, version 4.5) and Pearson correlation analysis (SPSS 17.0)" in the Statistical Analysis section.

52. Line 177-179 " The relationships between soil enzyme activities and soil environmental factors are shown in Fig. 4 (b). The RD1 and the second (RD2) ordination axes explained 50.1% and 19.9% of the total variability in the enzyme activities, respectively. The concentrations of DOC, NO_3^--N, NH_4^+-N were mainly related to RD2 ordination axis." Explain what does it suggest for the ecosystem, for forest nutrition. Boring results though

Response:

We used RDA to analysis the relationships between soil enzyme activities and soil abiotic properties. And the content of DOC was positively correlated with αG, and was negatively correlated with βX and AP. The content of NH_4^+-N was positively correlated with αG and βG ($P < 0.05$; Table A2).

53. Line 182-185 "Pearson correlation analysis demonstrated that…"Were these correlations significant?

Response:

The results of Pearson correlation we show in the text were significant, and we have added the $P < 0.05$ at the end of these sentences.

54. Line 188 "soil C" better term would be soil organic C.

Response:

We have changed "soil C (DOC, POC and SOC)" to "soil organic C (DOC, POC and SOC" throughout the manuscript.

55. Line 195-196 "Studies in the past have shown that a source of soil C and nutrients, such as rhizosphere secretions, fine root turnover (Liu et al., 2012) and the SOM decomposition rate (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), decline when the understory vegetation is removed". Repetition. The authors mentioned that already.

Response:

We have deleted this repeated sentences.

56. Line 199 N- supply by fir roots would explain more about? " root residue of understory vegetation" Explain here why could that happen?

Response:

Plant mainly secrete carbohydrate to soil thorough root. We have revised this sentence as “The increased quantities of C secreted by Chinese fir roots and originated from decomposition of understory vegetation root residues did not fully compensate for the C lost when understory vegetation was removed”.

57. Line 200 Delete “in this study”.

Response:

We have deleted “in this study”.

58. Line 202 If there are less plants around can it be that more N is available in the soil?

Response:

In our study soil total and ammonium N content decreased but nitrate N did not change after understory vegetation removal. We have deleted the sentence “Therefore, soil C and N concentrations may decrease by removing understory vegetation and reducing plant diversity” And we added the sentence “Previous study have found that the reduction of labile root C input resulted in the increment of soil N contents as a result of reduced plant N uptake (Kaiser et al., 2010; Loeppmann et al., 2016a). However, we found the N contents increased with understory vegetation intact, maybe because more labile C input from root exudates have resulted the accumulation of SOM and promoted the mineralization of organic N simultaneously”.

59. Line 204-206 “The changes in the POC concentrations indicated that understory vegetation intact improved soil sustainability and productivity in

Chinese fir forests, since aggregate stability and POC concentrations were related (Bouajila and Gallali, 2010)". This sentence is not clear. Delete or rephrase.

Response:

We have revised this sentence as " The decreased values of the POC/SOC ratios after understory vegetation removal (Table 1) suggest that POC declined more than SOC when understory vegetation was removed. This was indicated that intact understory vegetation improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC contents were related (Bouajila and Gallali, 2010). This also means that when understory vegetation was removed, the decomposition from POC to SOC could occur at higher rates".

60. Line 207 So soil evaporation is higher in the fir forest than the water uptake of the understory? Do you have data on that?

Response:

We did not have the data. We have revised the sentence to "In addition, the decrease in the SMC by understory vegetation removal (Table 1) reflects that understory vegetation had the ability to hold soil water.".

61. Line 208-209 "Consistent with our hypothesis, the microbial biomass, including total PLFAs, bacterial, and fungal PLFA biomarkers, declined after the understory vegetation was removed in this study (Fig. 2)". not clear: MBC just includes PLFA. So why then are talking about MBC? This sentence is repetition of your results.

Response:

We have revised the sentence as "Consistent with our hypothesis, total PLFAs,

including bacterial and fungal PLFA biomarkers declined after the understory vegetation was removed in this study (Fig. 2)". And we have revised "microbial biomass" to "PLFA content" throughout the manuscript.

62. Line 209 Delete "also".

Response:

We have delete "also", and revise the sentence to "Previous studies reported decreases in fungal biomass after understory vegetation removal…".

63. Line 212-216 Delete "In this study". "In our study, the decline in fungal biomass may reflect the decrease in plant diversity". Can you explain why you suggest that? Pure speculation! Have you checked for AMF? What then about EMF?

Response:

We have already analyzed the biomarker of 16:1w5, which is considered as the indicator of arbuscular mycorrhizal fungi (AMF). And we confirmed our speculation "The PLFA content of AMF was declined (P = 0.053) after understory vegetation removal (Fig, A1) which may reflect the influence of the reduction of plant diversity. Since specific AMF may only grow when specific plants are present, plant communities' change over time will change their mycorrhizal partners (Hart et al., 2001)".

64. Line 218-220 "Therefore, when the amounts of C and exuded by the rhizosphere decreased after the understory vegetation was removed, the soil fungal biomass also decreased, since soil fungi dominated decomposition of C in the rhizosphere (Denef et al., 2009)". incomplete sentence.

Response:

We have revised the sentence as “Compared with other fungi, mycorrhizal fungi depends highly on belowground C allocation by plants, thus, the reduction of fungal PLFA content was mainly related to the reduction of mycorrhizal fungi (Kaiser et al., 2010)”.

65. Line 222-223 “The F/B ratio did not change because the bacterial and fungal biomass decreased at the same time (Fig. 2)” This is result, shift.

Response:

We have moved this sentence to Results section.

66. Line 257-258 “The ratio of SOC/TN did not change…”. which might refer to higher SOM contents include lots of N. Microbial necromass might also be higher in forests compared to arable soil.

Response:

We have revised this sentence as “The rhizosphere of the understory vegetation was not N-limited because the ratios of SOC/TN did not change with higher SOM and TN contents relative to understory vegetation removal.”.

67. Line 259 “more SOM derived from root enhanced NAG activity” Would you please explain the reviewer, how a higher SOM may derive from the root?

Response:

We have deleted this sentence, and we have added “In line with Loeppmann et al. (2016b), the potential activity of NAG was lower, when the understory vegetation was removed. The lower potential NAG activity and less NH_4^+-N content after understory vegetation removal reflect that less root exudates might inhibit the

decomposition of organic N due to carbon limitation" according to comment 69 of Comments Attached of yours.

68. Line 261 "Chitin, a major structural component of fungal cell wall, can be degraded by NAG (Mganga et al., 2015)." it is also in in bacterial cells as peptidoglycan. REF eg. Loeppmann et al. 2016.

Response:

We have revised this sentence as " Chitin, a major structural component of fungal cell wall, and peptidoglycan, a major structural component of bacterial cell wall (Loeppmann et al., 2016b), can be degraded by NAG (Mganga et al., 2015)".

69. Line 262-264 "The activity of NAG was lower when the understory vegetation was removed than the understory vegetation intact, which might reflect a reduction in fungal biomass" clear, because less N competition for N because of less N uptake by plants, leading to more available N for fir and microbes which would be in line with Loeppmann et al., 2016 (Substrate quality affects microbial- and enzyme activities in rooted soil); Steinweg et al., 2013; Taylor et al., 2002.

Response:

"In line with Loeppmann et al. (2016b), the potential activity of NAG was lower, when the understory vegetation was removed. The lower potential NAG activity and less NH_4^+-N content after understory vegetation removal reflect that less root exudates might inhibit the decomposition of organic N due to carbon limitation".

70. Line 265-267 "The negative relationship between the activity of AP and the concentration of DOC indicated that microorganisms absorbed more C to meet the demands for P in the P limited area". could you explain these sentence. Why does

increasing DOC indicate higher microbial C incorporation?

Response:

We have revised this sentence as "The negative relationships between the potential activity of AP and the content of DOC indicated that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition" according to comment 12 of Comments Attached of yours.

71. Line 272-274 " The activity of AP among all the measured enzymes is the highest may reflect the P was limited in this area, while NAG was positive with the concentration of NO_3^--N, reflected that P- and N- degrading enzyme affected by different mechanism". See my comments to AP above.

Response:

We have deleted this sentence according to comment 10 of Comments Attached of yours.

Anonymous Referee #2:

The manuscript 'Understory vegetation plays a key role in sustaining soil microbial biomass and extracellular enzyme activities" by Yang and co-authors describes interesting findings and documents well the role of understory vegetation on soil nutrient dynamics, microbial community composition and extracellular enzyme activities.

1. The manuscript addresses relevant scientific questions within the scope of the journal, and the results are interesting, but the interpretation could be still a bit

more elaborated.

Response:

We have modified the Discussion section according to your comments.

We have deleted the repeated sentences “Studies in the past have shown that a source of soil C and nutrients, such as rhizosphere secretions, fine root turnover (Liu et al., 2012) and the SOM decomposition rate (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), decline when the understory vegetation is removed”; we have deleted the too far reached sentence “Our results suggest that bacterial and fungal biomass were better indicators of the changes in understory management practices in the Chinese fir plantation (arbuscular mycorrhizal species) than actinomycetes”.

We have revised the sentences “The decreased values of the POC/SOC ratio (Table 1) suggest that POC changed more than SOC when understory vegetation was removed. The changes in the POC concentrations indicated that understory vegetation intact improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC concentrations were related (Bouajila and Gallali, 2010).” to “The decreased values of the POC/SOC ratios after understory vegetation removal (Table 1) suggest that POC declined more than SOC when understory vegetation was removed. This was indicated that intact understory vegetation improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC contents were related (Bouajila and Gallali, 2010). This also means that when understory vegetation was removed, the decomposition from POC to SOC could occur at higher rates”.

We have revised all of the “microbial biomass” to “PLFA content”; we have already analyzed the biomarker of 16:1w5, which is considered as the indicator of arbuscular mycorrhizal fungi (AMF). And we confirmed our speculation “The PLFA content of AMF was declined (P = 0.053) after understory vegetation removal (Fig, A1) which may reflect the influence of the reduction of plant diversity. Since specific AMF may only grow when specific plants are present, plant communities’ change over time will change their mycorrhizal partners (Hart et al., 2001)”.

We have considered the publication by Kaiser et al 2010 on how belowground C allocation affects microbial dynamics to illustrate the possible reasons for the decreased enzyme activities after understory vegetation was removed; we have explained why AP was higher reflected the P limitation in the area, “In highly weathered red soil in southern China, P is the most limiting element, and most soil P is presented in organic form or is immobilized by high contents of Al and Fe (Margalef et al., 2017). Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was limiting nutrient in red soil. Soil microorganisms may produce more phosphatase to mineralized organic P and release phosphate to meet their demand for P (Allison and Vitousek, 2005)”.

2. The authors draw some comprehensible conclusion on the importance of understory vegetation to improve soil C sequestration. However they also conclude that high AP rates indicate P limitation, which, if they want to show it must be more elaborated (see. e.g. Margalef et al 2017, or Sinsabaugh et al 2008), and also it might be worth to compare the effect of the treatment on enzyme rates

normalized by microbial biomass C (or total PLFAs).

Response:

In highly weathered red soil in southern China, P is the most limiting element, and most soil P is presented in organic form or is immobilized by high contents of Al and Fe (Margalef et al., 2017). Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was limiting nutrient in red soil. Soil microorganisms may produce more phosphatase to mineralized organic P and release phosphate to meet their demand for P (Allison and Vitousek, 2005).

And we have analyzed the specific enzyme activities normalized by total PLFAs, as well as the stoichiometry of enzyme activity through calculating the ratios of C/N and C/P potential acquisition activity, as indicated by ratios of ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP, respectively.

3. Also the authors speculate that understory removal could have induced a shift in arbuscular (or other) mycorrhizal fungi composition, maybe it would be interesting to show more details on shifts in fungal marker composition (e.g. 16:1w5 compared to the other markers).

Response:

We analyzed the biomarker of 16:1w5, which was considered as the indicator of arbuscular mycorrhizal fungi (AMF). And we found that the PLFA content of AMF marginally declined after understory vegetation was removed (P = 0.053), which confirmed our speculation "Specific AMF may only grow when specific plants are present, plant communities' change over time will change their

mycorrhizal partners (Hart et al., 2001)”

4. There was also some temporal variation in PLFAs, so why not pay them more attention? The methods seem to be sound, but it would be helpful to state a bit more details on the RDA, were absolute PLFAs analyzed or group means, or relative marker composition? And were enzyme rates log transformed? More specific comments are in the supplement.

Response:

The data we used in the text was the average data of April, July and November. And we just present soil abiotic and biotic properties, such as PLFA contents, and extracellular enzyme activities in different months in the Supplementary Material (Table A4, A5 and A6).

The PLFA data we used was absolute PLFA data. We have made a matrix with individual PFLAs to illustrate the RDA, but the result was not well, so we use the group PLFAs of bacteria, fungi and actinobacteria.

We have calculated the soil potential enzyme activities, and we also have analyzed the specific enzyme activities normalized by total PLFAs in the modified version of the manuscript.

Comments Attached of Anonymous Referee #2:

1. Line 17 there is no treatment where nothing was changed (also no litter removal?)

Response:

Yes, there is no treatment where nothing was changed. We studied the effects of

understory vegetation on the soil abiotic and biotic properties and avoid the interference of litter by removing litter.

2. Line 43 "capacity to release nutrients to the soil". this is meant to be via the decomposition of understory derived litter?

Response:

We have revised "capacity to release nutrients to the soil" to "capacity to release carbohydrates to soil via the decompositions of understory derived litter and rhizodeposition".

3. Line 44 I would suggest to make a clear distinction between nutrient limitations for plants and microbes...

Response:

We have revised the sentence "Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Stone et al., 2014), in line with the nutrient requirements of plants and microorganisms to ensure the nutrient balance is maintained the context of the changes in soil environment (Burns et al., 2013)" to "Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Burns et al., 2013; Nannipieri et al., 2018). Individual enzyme activities reflect the nutrient requirements of microorganisms and the microbial strategies for maintaining the nutrient balance in response to changes in the soil environment (Burns et al., 2013)".

4. Line 47 Replace "is affect" with "is affected".

Response:

We have revised “is affect” to “is affected”.

5. Line 52 “respiration” which respiration flux? soil respiration or ecosystem respiration?

Response:

“respiration” was revised to “soil respiration”.

6. Line 54 The effects, rather than results?

Response:

“The results” was changed to “The effects”.

7. Line 64 sure it is NEP and not NPP?

Response:

We have checked the reference, and NEP is right.

8. Line 89 indicate soil classification system.

Response:

We have revised the sentence as “The main soil type in this area is red soil, which forms from red sandstone and sandy conglomerate and is classified as Udults using the USDA-NRCS soil taxonomy (1996)”.

9. Line 90 could you add some info about tree height, DBH or LAI.

Response:

“The average tree height and diameter at breast height (measured at 1.3 m above ground level) were about 18 m and 17 cm, respectively”. We did not measure the data of leaf area index and in the future, we will add this index.

10 Line 102-103 “hm^{-2}” is this hectometer? I would suggest to stick to the commonly used abbreviation: ha.

Response:

We have changed "hm^{-2}" to "ha^{-1}".

11. Line 106 was there a reason for collecting samples at three time points? e.g. dry/wet season or else?

Response:

We have revised the sentence " Soil samples were collected in April, July, and November 2015" to "Bulk soil samples were collected in wet season (April and November) and dry season (July) in 2015".

12. Line 111 Replace "drying at 105" with "drying aliquots of soil".

Response:

We have revised the sentence "The soil moisture content (SMC) was measured by drying at 105 ℃ to constant weight" to "The soil moisture content (SMC) was measured by drying aliquots of soil at 105 °C to constant weight".

13. Line 122 "actinomycetes". It would be better to use actinobacteria here, actinomycetes are one group of actinobacteria, and wouldb e gram positive bacteria. so they could even be counted to the bacterial biomass.

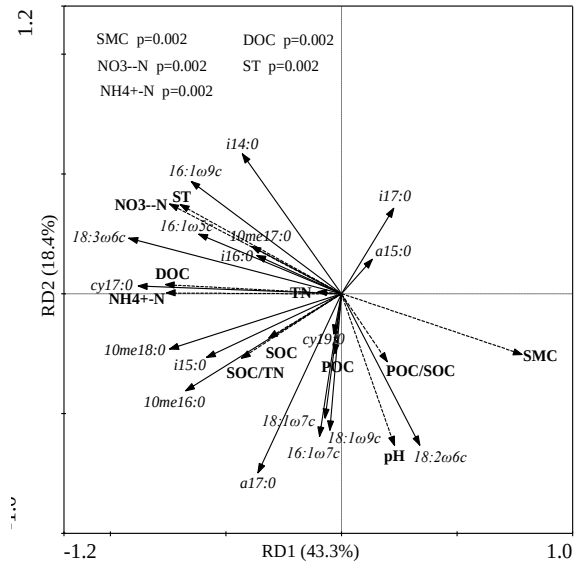
Response:

We have revised all "actinomycetes" to "actinobacteria" throughout the manuscript.

14. Line 142 "We investigated the relationships among soil environmental factors and different microbial biomass, and soil enzyme activities using redundancy analysis". it could be interesting to use individual PLFAs (or their relative abundances), instead of the groups ratios.

Response:

We have made a matrix with individual PFLAs to illustrate the RDA, but the result was not well, so we use the group PLFAs of bacteria, fungi and actinobacteria.



Redundancy analysis of all soil abiotic properties and individual PLFA contents

15. Line 155 here it would be interesting to see effects on fungi to bacteria ratios..., or state effects on microbial community composition. did you use total PLFA as microbial biomass?

Response:

We have added the result of fungi to bacteria ratios in the manuscript "The ratios of fungi/bacteria did not change because the bacterial and fungal PLFA contents decreased simultaneously when understory vegetation was removed". We have revised "microbial biomass" to "PLFA contents".

16 Line 162 "Some of the soil C- and N- hydrolase and oxidase activities were higher in the treatments with understory vegetation than in the treatment without understory vegetation (Fig. 3)". significantly?

Response:

It is significant. We have added the P values in the sentence as "Understory vegetation significantly affected soil enzyme activities. The potential activities of βG, NAG, PPO, and PER were higher in the treatments with understory vegetation than in the treatment without understory vegetation (Fig. 3a and b) ($P < 0.05$)".

17. Line 165 "While phosphate hydrolase activities in the Understory treatment were the same as in the None treatments ($P > 0.05$)" this sentence can't grammatically be standing alone, but has to be connected to the previous one.

Response:

We have modified this sentence as "When the understory vegetation was removed, the potential activities of βG, NAG, PPO, and PER reduced by 13%, 24%, 21% and 20%, respectively ($P < 0.05$), while the potential activity of acid phosphatases were not changed ($P > 0.05$)".

18. Line 169 I am not sure if it is very useful to put total PLFAs and the individual PLFAs in one ordination plot. I think it would be better to make a matrix with individual PFLAs and check if groups are affected differently.

Response:

We have made a matrix with individual PFLAs to illustrate the RDA, but the result was not well, so we use the group PLFAs of bacteria, fungi and actinobacteria. Please refer to comment 14 of Comments Attached of Anonymous of yours.

19 Line 170 did you use relative or absolute PLFA data?

Response:

The PLFA data we used was absolute PLFA data.

20. Line 177 enzyme stoichiometry could be useful to show limitations here. check

e.g. Sinsabaugh et al 2008 or Margalef et al 2017.

Response:

We have analyzed ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP. And we found that the soil C/N potential acquisition activity increased when understory vegetation is removed, which may mean that less labile C inputs are there led microbes to produce more enzymes comes at C cost relative to N cost (Kaiser et al., 2010).

21 Line 192 "the variety of understory vegetation species". species forming the understory vegetation, or understory vegetation composition.

22. Line 193 "influence of litter" you cannot test that with your experiment, as both treatments had litter removed, right?

Response:

Yes, both treatments had litter removed in our study. We have revised "The distinct results might largely depend on the variety of understory vegetation species in different studies (Nilsson and Wardle, 2005) and the influence of litter" to "The distinct results might largely depend on the understory vegetation compositions in different studies (Nilsson and Wardle, 2005)".

23. Line 204 "The changes in the POC concentrations" in which treatment was it decreased? "understory vegetation intact" change to "intact understory vegetation". this also means that when understory vegetation was removed that the decomposition from POC to SOC could occur at higher rates.

Response:

We have revised this sentence "The changes in the POC concentrations indicated

that understory vegetation intact improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC concentrations were related (Bouajila and Gallali, 2010)" to "The decreased values of the POC/SOC ratios after understory vegetation removal (Table 1) suggest that POC declined more than SOC when understory vegetation was removed. This was indicated that intact understory vegetation improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC contents were related (Bouajila and Gallali, 2010). This also means that when understory vegetation was removed, the decomposition from POC to SOC could occur at higher rates".

24 Line 213 did you check the marker dynamics of 16:1w5?

25 Line 214 "If plant communities change over time, their mycorrhizal partners will also change (Hart et al., 2001)". see newer citations.

Response:

We have analyzed the biomarker of 16:1w5, which was considered as the indicator of arbuscular mycorrhizal fungi. And we found that "The PLFA content of AMF was declined (P = 0.053) after understory vegetation removal (Fig, A1) which may reflect the influence of the reduction of plant diversity. Since specific AMF may only grow when specific plants are present, plant communities' change over time will change their mycorrhizal partners (Hart et al., 2001)".

26. Line 219 "the understory vegetation was removed, the soil fungal biomass also decreased". I am not sure if this is a causal relation... it could also be the other way around...

Response:

It is a causal relation because understory vegetation removal was a treatment. We have explained the reasons of understory vegetation removal decrease fungal PLFA contents. “The PLFA content of AMF was declined (P = 0.053) after understory vegetation removal (Fig, A1) which may reflect the influence of the reduction of plant diversity. Since specific AMF may only grow when specific plants are present, plant communities’ change over time will change their mycorrhizal partners (Hart et al., 2001). Compared with other fungi, mycorrhizal fungi depends highly on belowground C allocation by plants, thus, the reduction of fungal PLFA content was mainly related to the reduction of mycorrhizal fungi (Kaiser et al., 2010). Mycorrhizal species in the study area included understory vegetation, such as *Dicranopteris dichotoma*, *Vaccinium bracteatum*, *Loropetalum chinense*, and *Rhododendron*. Chinese fir (arbuscular mycorrhizal plant) monocultures may support fewer fungi biomass than other plantations where the understory vegetation is left intact”. And previous studies reported decreases in fungal biomass after understory vegetation removal (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013).

27. Line 226-227 “bacterial and fungal biomass were better indicators of the changes in understory management practices in the Chinese fir plantation (arbuscular mycorrhizal species) than actinomycetes”. this is a bit too far reached, see earlier comments, maybe different indicator markers would be better suited to make a point here.

Response:

We have deleted this too far reached sentence.

28 Line 229 "was agree with" change to "in line with".

Response:

"was agree with" was change to "in line with".

29. Line 229 "soil cellulose activity declined" a decline in cellulase activity (I guess is meant here).

Response:

" cellulose activity" was change to "cellulase activity".

30. Line 230 do not use didn't

Response:

We have revised the sentence "in spite of Lin et al., (2012) didn't found changes in soil enzyme activities" to "in spite of Lin et al., (2012) found no changes in soil enzyme activities".

31. Line 231 "has been described as" change to "is a hotspot of microbial activity".

Response:

We have revised the sentence of "The soil rhizosphere has been described as soil microbial hotspots with higher microbial activities than other areas of the soil profile (Kuzyakov and Blagodatskaya, 2015)" to "The soil rhizosphere is a hotspot of microbial activities (Kuzyakov and Blagodatskaya, 2015)".

32. Line 235 " (1) When understory vegetation is removed, less organic matters are released to the soil from the lower amounts of root (Liu et al., 2012), which means there will be less substrates available for enzyme production". improve a bit the working... may consider the publication by Kaiser et al 2010 on how

belowground C allocation affects microbial dynamics.

less labile C inputs are there, so microbes may need a different energy (C) source, and producing enzymes comes at a C cost.

Response:

We have revised this sentence to "(1) The soil C/N potential acquisition activity increased when understory vegetation is removed, which may mean that less labile C inputs are there led microbes to produce more enzymes comes at C cost relative to N cost (Kaiser et al., 2010)".

34. Line 252 "P was the most limiting nutrient in this acidic Chinese fir forest soil". it is very speculative to d discuss about P limitation, without having any P concentrations measured.

35. Line 253 "P is limited" check also the paper by Dijkstra et al 2013.

Response:

In highly weathered red soil in southern China, P is the most limiting element, and most soil P is presented in organic form or is immobilized by high contents of Al and Fe (Margalef et al., 2017). Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was limiting nutrient in red soil. Soil microorganisms may produce more phosphatase to mineralized organic P and release phosphate to meet their demand for P (Allison and Vitousek, 2005).

36. Line 259 did you test if you found more or less root biomass? how could you test this with experimental setup?

Response:

We did not analyze the root biomass. And we have deleted this sentence "The positive correlation between NAG activity and NO_3^--N concentrations in our study (Table A2) may suggest that more SOM derived from root enhanced NAG activity may in turns promote the mineralization of SOM, thereby increased soil available N concentrations" according to comment 10 of Comments Attached of Anonymous Referee #1. We have added the sentence "In line with Loeppmann et al. (2016b), the potential activity of NAG was lower, when the understory vegetation was removed. The lower potential NAG activity and less NH_4^+-N content after understory vegetation removal reflect that less root exudates might inhibit the decomposition of organic N due to carbon limitation" according to comment 69 of Comments Attached of Anonymous Referee #1.

37. Line 260 "promote the mineralization of SOM, thereby increased soil available N concentrations" differences in soil moisture could also favor conditions for nitrification, and might have nothing to do with NAG activity.

Response:

Combining your attached comments 36 with comment 69 of Comments Attached of Anonymous Referee #1, we have revised this sentence to "In line with Loeppmann et al. (2016b), the potential activity of NAG was lower, when the understory vegetation was removed. The lower potential NAG activity and less NH_4^+-N content after understory vegetation removal reflect that less root exudates might inhibit the decomposition of organic N due to carbon limitation".

38. "The negative relationship between the activity of AP and the concentration of DOC indicated that microorganisms absorbed more C to meet the demands for P

in the P limited area" what exactly was the P limited area? which of the treatments?

Response:

We meant that P is limited in both treatments in red soil. And we have revised this sentence to " The negative relationships between the potential activity of AP and the content of DOC indicated that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition" according to comment 12 of Comments Attached of Anonymous Referee #1.

Understory vegetation plays the key role on sustaining soil microbial biomass and extracellular enzyme activities

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Abstract:

Understory vegetation affects soil microbial biomass and extracellular enzyme activities in a subtropical Chinese fir (*Cunninghamia lanceolata*) forests. The aim of this study was to determine the role of understory vegetation in controlling soil abiotic and biotic properties, such as PLFAs contents, and extracellular enzyme activities. One paired treatment, which comprised understory vegetation removal (**None**) and understory vegetation left intact (**Understor**y) in the context of litter removal, was established in a subtropical Chinese fir plantation. We mainly evaluated the effects of understory vegetation on soil abiotic properties, the PLFA contents of bacteria, fungi and actinobacterias, and the activities of five hydrolases and two oxidative enzymes. The soil moisture content (SMC), contents of soil dissolved organic carbon (DOC), particulate organic carbon (POC), soil organic carbon (SOC), ammonia nitrogen (NH_4^+-N), total nitrogen (TN), and the POC/SOC ratios respectively declined by 4%, 18%, 25%, 12%, 34% and 12%, and soil bacterial, fungal and total PLFA contents, and the activities of β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase (NAG), phenol oxidase (PPO), as well as peroxidase (PER) were up to 27% lower, when the understory vegetation was removed. The soil ln(αG+βG+βX)/lnAP (βX: β-1,4-xylosidase; AP: acid phosphatase) increased when understory vegetation is removed, which may mean that less labile carbon (C) inputs led microbes to produce more enzymes comes at C cost relative to N cost. The positive relationships between DOC and AP implied that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition. The contents of NH_4^+-N were positively correlated with and βG suggested the increased availability of N promoted the decomposition of C. Understory vegetation alter soil microbial biomass, which may influence the decomposition of soil organic matter, by changing soil carbon inputs. We therefore propose that, to sustain soil quality in subtropical Chinese

fir plantations, understory vegetation should be maintained.

Keywords: Chinese fir forest; Red soil; Enzyme activities; Phospholipid fatty acids; Understory vegetation

1. Introduction

The interactions between above-ground vegetation functional groups and soil microbial community structures are thought to be important drivers of carbon (C) and nutrient cycling in terrestrial ecosystems (Murugan et al., 2014). Understory vegetation removal influence soil processes by reducing above-ground plant diversity (Lamb et al., 2011) and biomass (Fu et al., 2015) and changing under-ground rhizodeposition quality (Li et al., 2013) in forest ecosystems. While understory vegetation absorbs water and nutrients from soil (Wang et al., 2014), it also releases carbohydrates, such as sloughed-off root cap and border cells, mucilage and exudates through root (McNear Jr, 2013) and cellulose, hemicelluloses and lignin in the form of leaf litter (Loeppmann et al., 2016a, b), to soils. The net effect of understory vegetation on soil nutrients is decided by the balance between the understory vegetation's nutrient demand and its capacity to release carbohydrates to soil via the decompositions of understory derived litter and rhizodeposition. Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Burns et al., 2013; Nannipieri et al., 2018). Individual enzyme activities reflect the nutrient requirements of microorganisms and the microbial strategies for maintaining the nutrient balance in response to changes in the soil environment (Burns et al., 2013). To study the changes of enzyme activities with understory vegetation removal could reveal how microbial nutrient acquisition is affected by microbial biomass and soil nutrients.

The influences of understory vegetation on soil properties were closely related to climate, soil type, plant species, and how long the manipulations have been applied (Li et al., 2013; Nilsson and Wardle, 2005; Zhang et al., 2014). There is no consensus about how understory vegetation impacts the physical, chemical, and biological properties of forest soils. Various studies have reported that the litter decomposition rate, soil organic matter (SOM) content, and the soil respiration rate decreased when the understory vegetation was removed (Wang et al., 2011; Liu et al., 2012; Wang et al., 2014), while others reported that its removal had little influence on soil properties (Xiong et al., 2008; Zhao et al., 2011). The effects of understory vegetation on soil microbial biomass also varied. Wu et al., (2011) and Zhao et al., (2013) found that fungal biomass and the fungi to bacteria ratio (F/B) decreased in the absence of understory vegetation, while in contrast, Murugan et al., (2014) found that bacterial and saprophytic fungal biomass increased after understory vegetation was removed from eucalyptus plantations. In an alpine shrubland, the soil arbuscular mycorrhizal fungal biomass decreased five months after plant functional groups were removed, but this effect disappeared after seventeen months (Urcelay et al., 2009). There is inconsistent information currently available about the responses of soil enzyme

activities to understory vegetation, reporting that soil enzyme activities decreased in the subtropical alpine coniferous forest (Huang et al., 2014), or did not change under *Pinus sylvestris* var. *mongolica* plantation (Lin et al., 2012), when understory vegetation was removed.

The average net ecosystem productivity of Chinese subtropical forests (362 ± 39 g C m^{-2} yr^{-1}) is approximately 82.6% and 64.9% higher than that of tropical and temperate forests, respectively (Yu et al., 2014). To maintain soil fertility it is important to ensure that C sinks and forest growth are sustained in these forests. Because of its high economic value, Chinese fir (*Cunninghamia lanceolata*) plantations are widespread in southern China. They cover an area of 9.11 × 10^6 ha, and account for approximately 18% of the total plantation area in China (Huang et al., 2013). To facilitate seed germination, ensure survival of seedlings, avoid the intense competition between understory vegetation and trees for water, nutrients and light, or for fuel, understory vegetation and litter were commonly removed from the forest floor in southern China and elsewhere (Xiong et al., 2008; Wu et al., 2011; Liu et al., 2012). As a shallow-rooted and fast-growing tree species, the Chinese fir competes intensively with understory vegetation for soil nutrients and moisture (He et al., 2015). It is still of high interest how the soil enzyme activities are affected by the understory vegetation removal in Chinese fir plantations.

In this study, we established a long-term field experiment to assess how understory vegetation influences soil abiotic properties, PLFA contents and enzyme activities at Chinese fir plantations. Earlier studies reported that the labile C release from below-ground C input decreased when understory vegetation was removed (Liu et al., 2012). We hypothesized that the removal of understory vegetation decreased rhizodepostion and therefore microbial biomass and activity. The interactions between soil abiotic and biotic properties under different forest understory management practices could gain new insights on forest nutrition.

2. Material and Methods

2.1 Experimental treatments

The study site was located at the Shixi forest plantation in Taihe County, Jiangxi Province, China (115°03′29.9″ E, 26°44′29.1″ N). The plantation experiences a subtropical monsoon climate with a mean annual temperature and precipitation of 18.8 ℃ and 1340 mm, respectively. The main soil type in this area is red soil (Munsell values: moisture, 7.5 YR 5/6 and dry, 7.5 YR 6/6), which forms from red sandstone and sandy conglomerate and is classified as Udults using the USDA-NRCS soil taxonomy (Soil Survey Staff, 1996).

The study site is a second-generation Chinese fir plantation that was planted in 1998. The average tree height and diameter at breast height (measured at 1.3 m above ground level) were about 18 m and 17 cm, respectively. The

understory vegetation, including shrubs and herbs, is dominated by Old World forked fern (*Dicranopteris dichotoma Berth*), gambir (*Uncaria*), oriental blueberry (*Vaccinium bracteatum*), Nutgall Tree (*Rhus chinensis*), Chinese witch hazel (*Loropetalum chinense*), short shank robe oak (*Quercus glandulifera BI.*), root of mayflower glorybower (*Clerodendron cyrtophyllum Turcz*), and andazalea (*Rhododendron*).

Three 30 m × 30 m plots, with a buffer zone between them exceeding 10 m to avoid the influence between each plot, were established in the Chinese fir plantation in January 2013. One paired treatment with three replications was established within each of the three plots. Each plot was divided into four 15 m × 15 m subplots and contained two treatments: understory vegetation and litter removal (**None**) and understory vegetation left intact but litter removal (**Understory**). The two subplots with the same treatment in one plot were distributed across each plot to avoid the effects of slope (Fig. 1) and were averaged as one analysis replication. The litter and understory were managed on a monthly basis. For the **None** treatment, we removed all litter and understory vegetation from the plot. For the **Understory** treatment, we removed the litter from the plot, but left the understory vegetation intact. The amount of litter was about 1020 kg ha^{-1} $year^{-1}$, and the amount of understory vegetation in the research site was about 6236 kg ha^{-1} under natural conditions.

2.2 Soil sampling and analysis

Bulk soil samples were collected in wet season (April and November) and dry season (July) in 2015. Five soil cores with an inner diameter of 5 cm were collected randomly from a depth of 0–10 cm in each subplot and then mixed as one composite sample. All fresh soil samples were sieved through a 2-mm mesh, stored at 4 ℃ prior to analysis.

Soil physical and chemical properties were determined as outlined by Bao (2008). Soil temperature (ST) was determined at a depth of 10 cm with a soil thermometer (TP101) when sampling. The soil moisture content (SMC) was measured by drying aliquots of soil at 105 ℃ to constant weight. Soil pH was measured at a soil to water ratio of 1: 2.5 by a pH digital meter. Soil nitrate N (NO_3^--N) and ammonia N (NH_4^+-N) contents were measured with a continuous flow analyzer (Bran Luebbe, AA3) after extraction with 2 *M* KCl solution (soil: solution ratio of 1: 10). Dissolved organic carbon (DOC) contents were measured with a TOC analyzer (Elementar, Liquid *II*) after extraction with ultra-pure water (soil: solution ratio of 1: 5) (Jones and Willett., 2006). Particulate organic carbon (POC) was determined as outlined in the method of Garten et al., (1999). Soil organic C (SOC) and total nitrogen (TN) contents were measured with an elemental analyzer (Vario Max CN).

Soil phospholipid fatty acids (PLFAs) were extracted following the procedure outlined by Bossio and Scow (1998), and were determined with a gas chromatograph (Agilent 6890N). Soil total PLFAs were represented by the following

PLFA biomarkers: gram positive bacteria (G^+: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), gram negative bacteria (G^-: 16:1ω7c, cy17:0, 16:1ω9c, cy19:0), fungi (arbuscular mycorrhizal fungi (AMF, 16:1ω5), 18:1ω9c, 18:2ω6c, 18:3ω6c), actinobacterias (10Me16:0, 10Me17:0, 10Me18:0); G^+ and G^- bacterial PLFA contents represented total bacterial PLFA contents (Bradley et al., 2007; Denef et al., 2009).

Soil enzyme activities were measured following the methods of Saiya-Cork et al., (2002). The specific substrates and functions of the enzymes assayed are listed in Table A1. Five hydrolase activities (α-1,4-glucosidase, β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase (βX) and acid phosphatase (AP)) were assayed using fluorogenically-labeled substrates. Briefly, a soil suspension was prepared by adding 1 g of fresh soil to 125 mL of 50 m*M* acetate buffer. We added 200 μL of the soil suspension and 50 μL of the substrate solution (200 μ*M*) to 96 microplates in eight analytical replicates. Methylumbelliferone (MUB) was used for calibration of hydrolase activities. The microplates were incubated in the dark at 20 ℃ for up to 4 h. After incubation, 10 μL of 1 M NaOH was added to each well to terminate enzymatic reaction. Following termination of each reaction, the fluorescence was measured using a microplate fluorometer (SynergyH4, BioTek) with excitation and emission filters of 365 nm and 450 nm, respectively.

The soil oxidase activities (polyphenol oxidase (PPO) and peroxidase (PER)) were assayed with spectrophotometrically. We added 600 μL of the soil suspension and 150 μL of the substrate solution to deep-well plates. We also added 30 μL of 0.3% H_2O_2 solution before determining PER. After incubation in the dark at 20 ℃ for up to 5 h, the deep-well plates were centrifuged for 3 minutes at 3000 r h^{-1}. We then moved 250 μL of the supernatant to the microplates and measured the absorbance at 450 nm with a microplate fluorometer (DeForest, 2009). We had eight replicate sample wells for each assay.

2.3 Statistical Analysis

Data we used were the average data of April, July and November. N=18, n=3. All of the data satisfy the normal distribution criteria for parameter analysis was tested by one-sample Kolmogorov-Smirnov test using SPSS 17.0. The differences of soil abiotic properties, PLFA contents and enzyme activities between the understory treatments were assessed by a paired-sample *t*-test (SPSS 17.0). Data from the two subplots with the same treatment in one plot were averaged and then analyzed statistically (n=3). We investigated the relationships among soil abiotic properties and PLFA contents and enzyme activities of all soil using redundancy analysis (RDA, CANOCO, version 4.5) and Pearson correlation analysis (SPSS 17.0). Monte Carlo Permutation Test was used to test the significance of the variables before conducted RDA. Figures were generated with SigmaPlot (Version 10.0). The significance level was $P < 0.05$.

3. Results

3.1 Soil abiotic properties

Soil C and N contents and the SMC were decreased, when understory vegetation was removed (Table 1). The contents of various soil organic C (including DOC, POC, and SOC) and N (including NH_4^+-N and TN) fractions, SMC and POC/SOC ratios were respectively 4%, 18%, 25%, 12%, 34% and 12% lower in the **None** treatment than in the **Understory** treatment ($P < 0.05$). The contents of NO_3^--N, ST, pH, and SOC/TN did not differ significantly between the **None** and the **Understory** treatment ($P > 0.05$).

3.2 Soil PLFA contents

Soil total PLFA contents were 27% lower in the **None** treatment than in the **Understory** treatment (Fig. 2). In specific, bacterial PLFA content was 26% less in the **None** treatment than in the **Understory** treatment ($P < 0.05$), though the PLFA contents of G^+ and G^- did not vary ($P > 0.05$). Soil fungal PLFA content was 20% lower in the **None** treatment than in the **Understory** treatment ($P < 0.05$). The ratios of fungi/bacteria did not change because the bacterial and fungal PLFA contents decreased simultaneously when understory vegetation was removed. Understory vegetation removal did not change actinobacterial PLFA contents as well ($P > 0.05$).

3.3 Soil enzyme activities

Understory vegetation significantly affected soil enzyme activities. The potential activities of βG, NAG, PPO, and PER were higher in the treatments with understory vegetation than in the treatment without understory vegetation (Fig. 3a and b) ($P < 0.05$). When the understory vegetation was removed, the potential activities of βG, NAG, PPO, and PER reduced by 13%, 24%, 21% and 20%, respectively ($P < 0.05$), while the potential activity of acid phosphatases were not changed ($P > 0.05$). Soil C/N and C/P potential acquisition activity was indicated by the ratios of ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP (Fig. 3c). The ratios of ln(αG+βG+βX)/lnNAG increased by 6.0%, while the ratios of ln(αG+βG+βX)/lnAP was not changed after understory vegetation was removed.

The trends were enzyme-specific when normalized by total PLFAs (Fig. 3d and e). The specific activities of C hydrolase (αG_{PLFAs}, βG_{PLFAs} and βX_{PLFAs}) significant increased after understory vegetation removal ($P < 0.05$), while the specific activities of N (NAG_{PLFAs}) and P hydrolase (AP_{PLFAs}) were not changed ($P > 0.05$).

3.4 Correlations between soil enzyme activities, soil PLFA contents, and soil abiotic properties

The relationships between different PLFA contents and soil abiotic properties are shown in Fig. 4 (a). The first (RD1) ordination axis explained 62.0% of the total variability in the different PLFA contents and was mainly correlated with ST, SMC, NO_3^--N, NH_4^+-N, DOC, SOC and SOC/TN, and the second (RD2) ordination axis explained 15.5% of the total variability in the different PLFA contents. The contents of NH_4^+-N and DOC were positively correlated with bacterial, actinobacterial and total PLFAs. The content of SOC was positively correlated with G^-, bacterial, fungal and total PLFAs. ($P < 0.05$) (Table A2).

The relationships between soil potential enzyme activities and soil abiotic properties are shown in Fig. 4 (b). The RD1 and the second (RD2) ordination axes explained 50.1% and 19.9% of the total variability in the potential enzyme activities, respectively. The contents of DOC, NO_3^--N, NH_4^+-N were mainly related to RD2 ordination axis. The content of DOC was positively correlated with αG, and was negatively correlated with βX and AP. The content of NH_4^+-N was positively correlated with αG and βG ($P < 0.05$; Table A2). Pearson correlation analysis demonstrated that bacterial and total PLFAs were positively correlated with αG, βG, NAG, PPO and PER. The PLFA content of fungi was positively correlated with αG, βG, NAG ($P < 0.05$; Table A3).

4. Discussion

Consistent with our hypothesis, the contents of soil organic C (including DOC, POC, and SOC) and N (including NH_4^+-N and TN) were decreased when the understory vegetation was removed (Table 1), which demonstrated that understory vegetation is beneficial to improve the content and availability of soil C and N. Other studies however reported that the responses of soil physical and chemical properties to understory vegetation removal were minimal (Xiong et al., 2008; Zhao et al., 2011). The distinct results might largely depend on the understory vegetation compositions in different studies (Nilsson and Wardle, 2005). In our study, we removed litter in all treatments to avoid the effects of litter. Although Chinese fir roots may occupy the space vacated and may partly compensate for the reduced C inputs by increasing their exudation (Li et al., 2016), understory vegetation root residue also incorporated into soil (Li et al., 2013) after understory vegetation removal. The increased quantities of C secreted by Chinese fir roots and originated from decomposition of the understory vegetation root residues did not fully compensate for the C lost when understory vegetation was removed. Additionally, soil C tends to be higher when plant functional diversity is high (Zhou et al., 2016). Therefore, soil C content may decrease by removing understory vegetation and reducing plant diversity. Previous study have found that the reduction of labile root C input resulted in the increment of soil N contents as a result of reduced plant N uptake (Kaiser et al., 2010; Loeppmann et al., 2016a). However, we found the N contents

increased with understory vegetation intact, maybe because more labile C input from root exudates have resulted the accumulation of SOM and promoted the mineralization of organic N simultaneously. The decreased values of the POC/SOC ratios after understory vegetation removal (Table 1) suggest that POC declined more than SOC when understory vegetation was removed. This was indicated that intact understory vegetation improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC contents were related (Bouajila and Gallali, 2010). This also means that when understory vegetation was removed, the decomposition from POC to SOC could occur at higher rates. In addition, the decrease in the SMC by understory vegetation removal (Table 1) reflects that understory vegetation had the ability to hold soil water.

Consistent with our hypothesis, total PLFAs, including bacterial and fungal PLFA biomarkers declined after the understory vegetation was removed in this study (Fig. 2). Previous studies reported decreases in fungal biomass after understory vegetation removal (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013). The PLFA content of AMF was declined (P = 0.053) after understory vegetation removal (Fig, A1) which may reflect the influence of the reduction of plant diversity. Since specific AMF may only grow when specific plants are present, plant communities' change over time will change their mycorrhizal partners (Hart et al., 2001). Compared with other fungi, mycorrhizal fungi depends highly on belowground C allocation by plants, thus, the reduction of fungal PLFA content was mainly related to the reduction of mycorrhizal fungi (Kaiser et al., 2010). Mycorrhizal species in the study area included understory vegetation, such as *Dicranopteris dichotoma*, *Vaccinium bracteatum*, *Loropetalum chinense*, and *Rhododendron*. Chinese fir (arbuscular mycorrhizal plant) monocultures may support fewer fungi biomass than other plantations where the understory vegetation is left intact. The bacterial biomass also decreased after the understory vegetation was removed, which was mainly the result of reductions in the soil C and N contents (Table A2) and plant diversity (Lamb et al., 2011). Brant et al., (2006) considered that there might be an increase in the biomass of actinobacterias to decompose recalcitrant C compounds when nutrient availabilities were low; however, we did not observe this pattern in our research (Fig. 2), perhaps because of the high variability in the actinobacterial PLFA content in the field plots.

Consistent with our hypothesis, we found a lower potential extracellular enzyme activity when understory vegetation was removed (Fig. 3), which was in line with the results of Huang et al., (2014), who found soil potential cellulase activity decline after understory vegetation removal, in spite of Lin et al., (2012) found no changes in soil enzyme activities. The soil rhizosphere is a hotspot of microbial activities (Kuzyakov and Blagodatskaya, 2015). Decreases in the quantity and diversity of root exudates in the understory vegetation, and changes in the soil abiotic and biotic properties, may cause direct and indirect changes in soil enzyme activities (Liu et al., 2012; Huang et al., 2014). The potential C hydrolase activity increased while the specific C hydrolase activities normalized by PLFAs decreased

with understory vegetation intact, which may reflected that more labile C input may led to the emergence of opportunistic microorganisms (the microorganisms that do not produce enzymes but use enzyme products) (Allison, 2005). There are several possible reasons for the changed enzyme activities observed in our study, as follows. (1) The soil C/N potential acquisition activity increased when understory vegetation is removed, which may mean that less labile C inputs are there led microbes to produce more enzymes comes at C cost relative to N cost (Kaiser et al., 2010). (2) Mycorrhizal fungi vanish when understory vegetation is removed (Fekete et al., 2011), which means there are fewer microorganisms to produce less enzymes. (3) For the understory vegetation remaining and removal treatment, continuous root exudates and discontinuous root residue were incorporated into the soil, respectively (Li et al., 2013). The different chemical composition of SOM sources may have different influence on enzyme activities.

We observed positive relationships between the activities of αG, βG and the contents of soil inorganic N fractions (Table A2), which reflected that the decreased availability of N reduced the decomposition of C when understory vegetation was removed. The size of soil C pool is the balance between the inputs and outputs of C (De Deyn et al., 2008). When understory vegetation is removed, both the soil C inputs, including root exudates, fine root turnover (Liu et al., 2012), and SOM decomposition rate (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), and soil C outputs, such as soil respiration (Wang et al., 2013), decrease. The decreased contents of SOC and TN caused by understory vegetation removal therefore indicate that the removal of understory vegetation had more effect on the outputs than inputs of soil C and N. Polyphenols are mainly decomposed by PPO, so the decrease in PPO activity may result in an increase in the content of polyphenols that have toxic effects on soil microbes and inhibit hydrolase activities (Sinsabaugh, 2010).

In highly weathered red soil in southern China, P is the most limiting element, and most soil P is presented in organic form or is immobilized by high contents of Al and Fe (Margalef et al., 2017). Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was limiting nutrient in red soil. Soil microorganisms may produce more phosphatase to mineralized organic P and release phosphate to meet their demand for P (Allison and Vitousek, 2005). The results of Loeppmann et al., (2016a) suggest that the same mechanism applies to N demand in the rhizosphere, as they found that N-degrading enzymes increased when N was limited in the rhizosphere of maize-planted soil. However, we did not find evidence that N demand is controlled by such a mechanism in this paper. The rhizosphere of the understory vegetation was not N-limited because the ratios of SOC/TN did not change with higher SOM and TN contents relative to understory vegetation removal. In line with Loeppmann et al. (2016b), the potential activity of NAG was lower, when the understory vegetation was removed. The lower potential NAG activity and less NH_4^+-N content after understory vegetation removal reflect that less root exudates might inhibit

the decomposition of organic N due to carbon limitation. Chitin, a major structural component of fungal cell wall, and peptidoglycan, a major structural component of bacterial cell wall (Loeppmann et al., 2016b), can be degraded by NAG (Mganga et al., 2015). We also found that there was a significant positive correlation between NAG and fungus biomass (Table A3). The potential activity of NAG was lower when the understory vegetation was removed than the understory vegetation intact, which might reflect a reduction in fungal biomass. We did not observe any change in AP activities when the understory vegetation was removed, perhaps because Chinese firs, along with their mycorrhizal associates, are the main producers of these enzymes. The negative relationships between the potential activity of AP and the content of DOC indicated that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition.

5. Conclusions

Our results demonstrate that understory vegetation plays an important role in enhancing soil potential C- and N-hydrolase and oxidase activities, but does not influence or P-hydrolase activity. The soil C/N potential acquisition activity increased after understory vegetation removal may imply that less labile C inputs are there led microbes to produce more enzymes comes at C cost relative to N cost. The positive relationships between the activities of C-degrading enzymes and the contents of soil inorganic N implied that the decreased availability of N inhibited the decomposition of C when understory vegetation was removed. The potential activity of AP is positive with the content of DOC indicated that increased DOC contents may increase P acquisition by increasing microbial biomass. Therefore, understory vegetation alter soil microbial biomass, which may influence the decomposition of soil organic matter, by changing soil C inputs. From this study, we can conclude that understory vegetation are beneficial for sustaining soil microbial activities in subtropical Chinese fir forests. We suggest that, as part of routine forestry management, understory vegetation should not be removed from, but rather should be maintained in, subtropical Chinese fir plantations.

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Figure captions

Fig. 1 One paired plot design treatments. Understory vegetation was either cut and removed (**None**) or left intact (**Understory**) in the context of removing litter.

Fig. 2 Soil phospholipid fatty acid (PLFAs) contents

(a) Soil PLFA contents, (b) ratio of PLFA contents. *None* **None**, *U* **Understory**, G^+/G^- ratio of gram positive bacteria to gram negative bacteria, F/B ratio of fungi to bacteria. Different lowercases represent significant differences among the **None** and **Understory** treatments ($P < 0.05$). Data was the average data of April, July and November. N=18, n=3. The same below

Fig. 3 Soil enzyme activities

(a) soil potential hydrolase activities, (b) soil potential oxidase activities, (c) Soil C/N and C/P potential acquisition activity was indicated by the ratios of ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP, (d) soil hydrolase activities normalized by total PLFAs. *αG* α-1,4-glucosidase, *βG* β-1,4-glucosidase, *NAG* β-1,4-N-acetylglucosaminidase, *βX* β-1,4-xylosidase, *AP* acid phosphatase, *PPO* phenol oxidase, *PER* peroxidase.

Fig. 4 Redundancy analysis of all soil abiotic properties and (a) PLFA contents, and (b) potential enzyme activities

SMC soil moisture content, *pH* soil pH, NO_3^--*N* soil nitrate nitrogen, NH_4^+-*N* soil ammonia nitrogen, *TN* soil total nitrogen, *DOC* soil dissolved organic carbon, *POC* soil particulate organic carbon, *SOC* soil organic carbon, *POC*/*SOC* ratio of POC to SOC, *SOC*/*TN* ratio of SOC to TN

Table captions

Table 1 Soil abiotic properties

Supplementary material

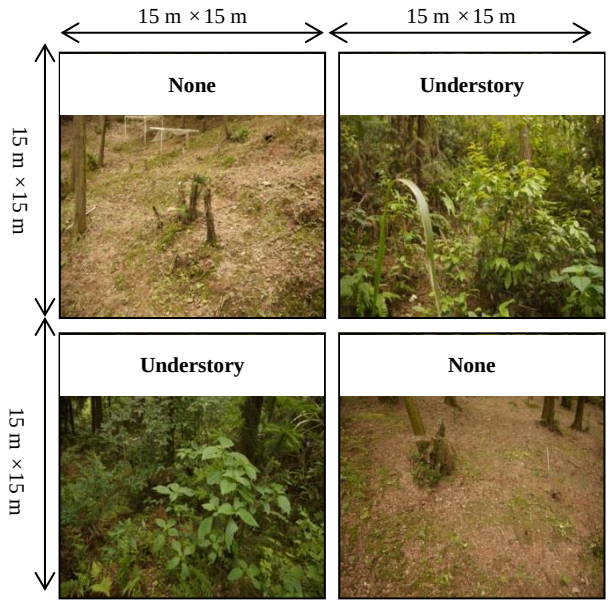
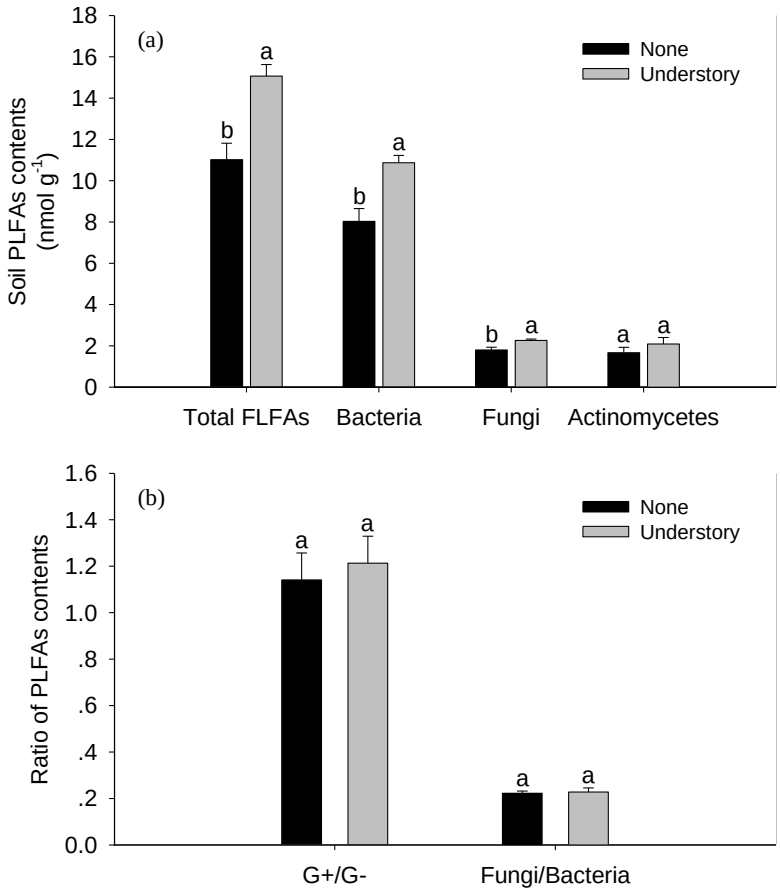

15 m ×15 m
15 m ×15 m
None
Understory
15 m ×15 m
Understory
None
15 m ×15 m

Fig. 1



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509 Fig. 2

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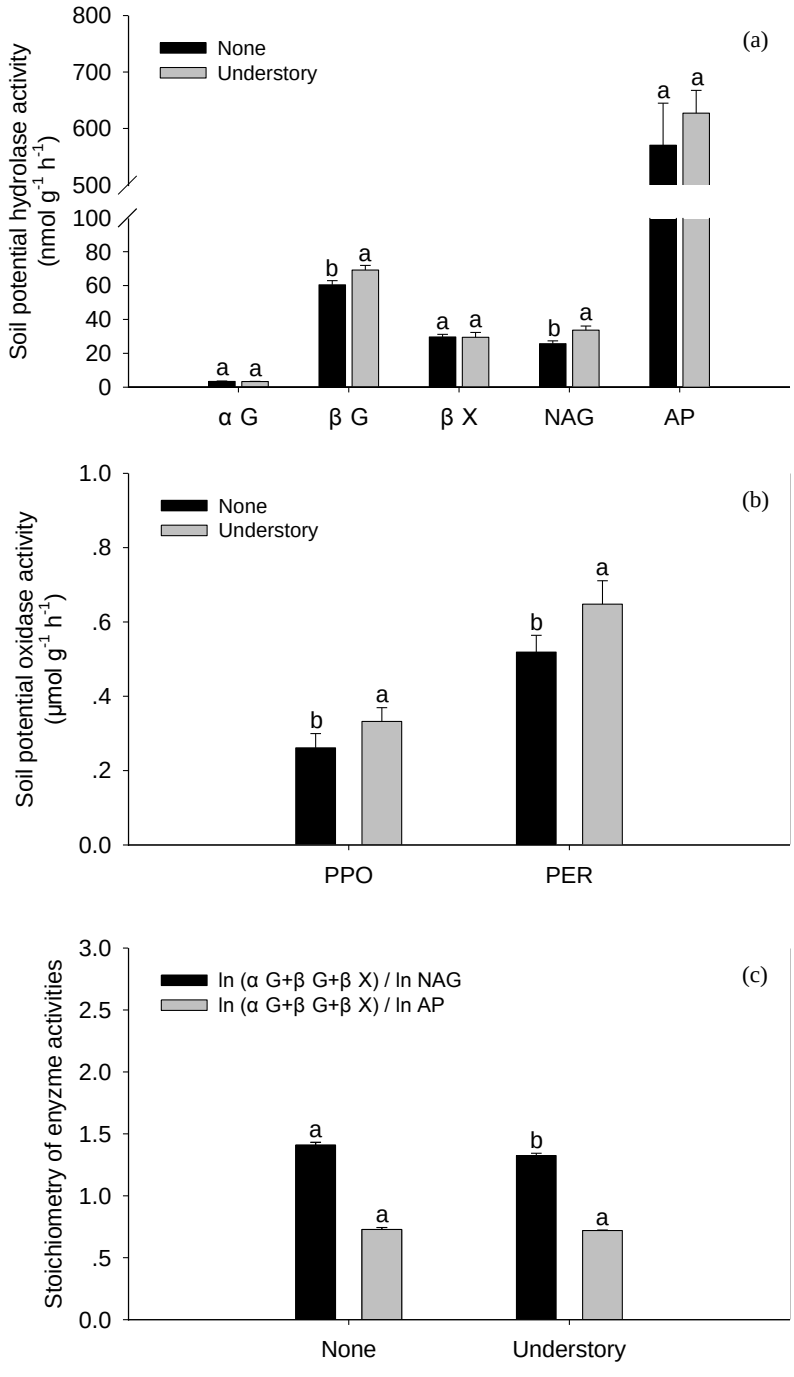
(a)
None
Understory
Soil potential hydrolase activity (nmol g-1 h-1)
800
700
600
500
100
80
60
40
20
0
a a
b a
a a
b a
a a
α G
β G
β X
NAG
AP
(b)
None
Understory
Soil potential oxidase activity (μmol g-1 h-1)
1.0
.8
.6
.4
.2
0.0
b a
b a
PPO
PER
(c)
ln (α G+β G+β X) / ln NAG
ln (α G+β G+β X) / ln AP
Stoichiometry of enyzme activities
3.0
2.5
2.0
1.5
1.0
.5
0.0
a a
b a
None
Understory

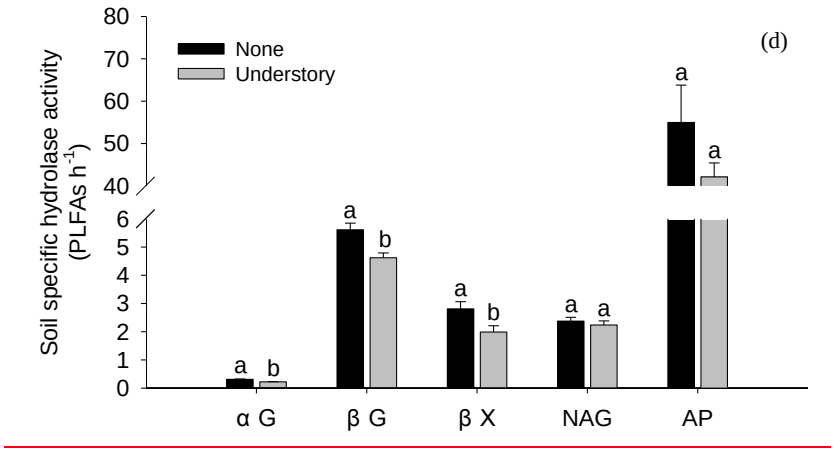
(d)
None
Understory
Soil specific hydrolase activity (PLFAs h-1)
80
70
60
50
40
6
5
4
3
2
1
0
a
b
a
b
a
b
a
a
a
a
α G
β G
β X
NAG
AP

Fig. 3

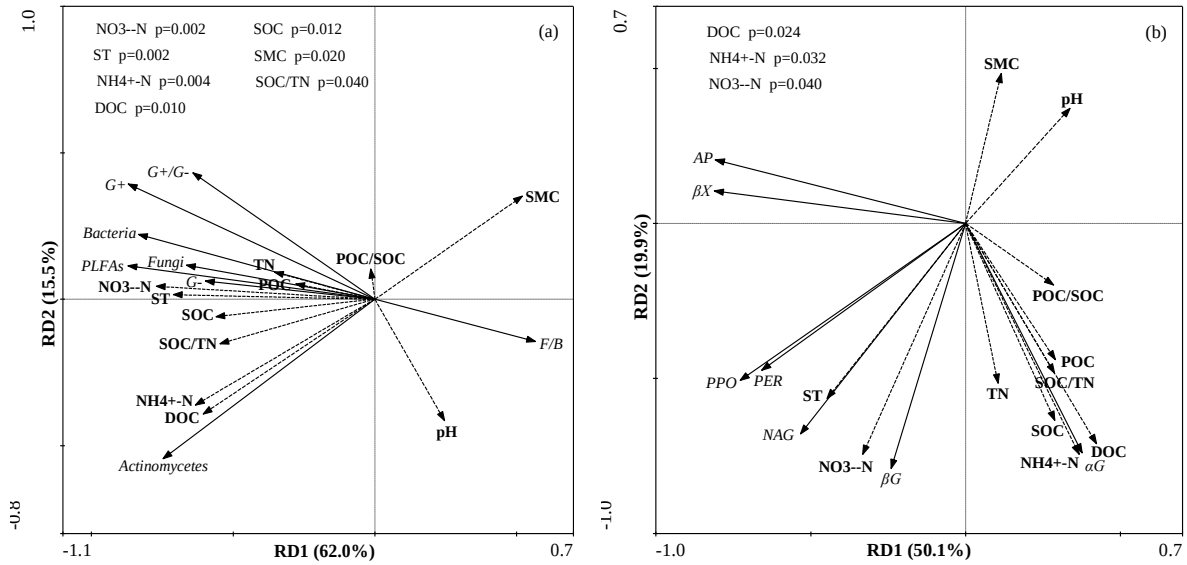
(a)
NO3--N p=0.002
ST p=0.002
NH4+-N p=0.004
DOC p=0.010
SOC p=0.012
SMC p=0.020
SOC/TN p=0.040
G+
G+/G-
Bacteria
PLFAs
Fungi
G-
NO3--N
ST
TN
POC
SOC
SOC/TN
POC/SOC
SMC
F/B
NH4+-N
DOC
pH
Actinomycetes
RD2 (15.5%)
RD1 (62.0%)
1.0
-0.8
-1.1
0.7
(b)
DOC p=0.024
NH4+-N p=0.032
NO3--N p=0.040
SMC
pH
AP
βX
POC/SOC
POC
SOC/TN
TN
SOC
DOC
NH4+-N
αG
PPO
PER
ST
NAG
NO3--N
βG
RD2 (19.9%)
RD1 (50.1%)
0.7
-1.0
-1.0
0.7

Fig. 4

Table 1 Soil abiotic properties

Treatment	ST (℃)	SMC (%)	pH	DOC (mg kg^{-1})	POC (mg kg^{-1})	SOC (g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TN (g kg^{-1})	POC/SOC (%)	SOC/TN
None	21.1±1.8a	21.92±0.9b	4.88±0.03a	37.3±3.4b	3.7±0.3b	17.6±0.8b	4.84±0.6a	14.72±2.5b	1.19±0.04b	20.6±1.0b	14.9±0.4a
Understory	21.0±1.7a	22.92±1.0a	4.87±0.03a	45.4±4.9a	4.9±0.3a	20.0±0.4a	5.50±0.5a	22.25±3.7a	1.30±0.01a	24.2±1.1a	15.4±0.3a

Values in the table are mean ± standard error. *ST* soil temperature, *SMC* soil moisture, *pH* soil pH, NO_3^-*-N* soil nitrate nitrogen, NH_4^+*-N* soil ammonia nitrogen, *TN* soil total nitrogen, *DOC* soil dissolved organic carbon, *POC* soil particulate organic carbon, *SOC* soil organic carbon, *POC*/*SOC* ratio of POC to SOC, *SOC*/*TN* ratio of SOC to TN. Different lowercase letters represented significant difference between **None** and **Understory** treatments ($P < 0.05$). Data was the average data of April, July and November. N=18, n=3. The same below

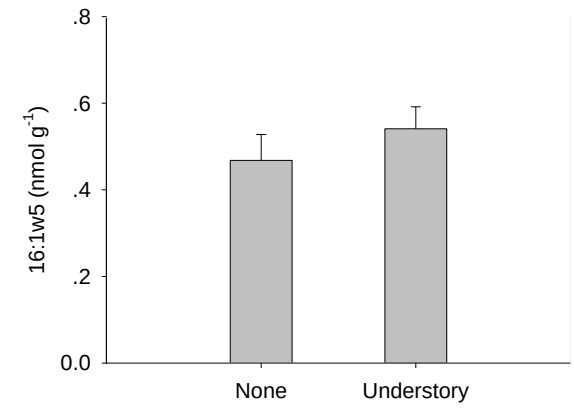

Fig. A1

595 Table A1 Soil enzymes and their corresponding substrates and functions

Enzyme	E. C	Abbreviation	Substrate	Function
Peroxidase	1.11.1.7	PER	L-DOPA	Oxidize lignin and aromatic compounds using H_2O_2 or secondary oxidants as an electron acceptor (Sinsabaugh 2010).
Phenol oxidase	1.10.3.2	PPO	L-DOPA	Oxidize phenolic compounds using oxygen as an electron acceptor (Sinsabaugh 2010).
α-1,4-glucosidase	3.2.1.20	αG	4-MUB-α-D-glucoside	Releases glucose from starch (Stone et al. 2014).
β-1,4-glucosidase	3.2.1.21	βG	4-MUB-β-D-glucoside	Releases glucose from cellulose (Stone et al. 2014).
β-1,4-xylosidase	3.2.1.37	βX	4-MUB-β-D-xyloside	Releases xylose from hemicellulose (Stone et al. 2014).
β-1,4-N -acetylglucosaminidase	3.2.1.14	NAG	4-MUB-N-acetyl-β-D -glucosaminide	Releases N-acetyl glucosamine from oligosaccharides (Stone et al. 2014).
Acid phosphatase	3.1.3.1	AP	4-MUB-phosphate	Releases phosphate groups (Stone et al. 2014).

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Table A2 Pearson correlation analysis of soil abiotic properties and different PLFA contents and potential enzyme activities

Abiotic Properties		ST	SMC	pH	NO_3^--N	NH_4^+-N	TN	DOC	POC	SOC	POC/SOC	SOC/TN
PLFAs	G^+	0.77**	-0.45	-0.38	0.72**	0.28	0.11	0.24	0.06	0.26	-0.13	0.39
	G^-	-0.05	0.15	-0.01	0.18	0.38	0.70**	0.27	0.52**	0.68**	0.33	0.29
	Bacteria	0.44	-0.24	-0.25	0.58*	0.62**	0.53*	0.57*	0.48*	0.65**	0.27	0.46
	Fungi	0.11	-0.02	-0.20	0.40	0.43	0.68**	0.39	0.56*	0.72**	0.38	0.36
	Actinobacterias	0.65**	-0.67**	-0.13	0.69**	0.69**	0.22	0.63**	0.08	0.36	-0.14	0.37
	PLFAs	0.54*	-0.37	-0.26	0.69**	0.63**	0.47*	0.60**	0.41	0.58*	0.20	0.43
	G+/G-	0.88**	-0.57*	-0.40	0.71**	0.14	-0.17	0.18	-0.17	-0.02	-0.29	0.25
	F/B	-0.50*	0.22	-0.01	-0.30	-0.17	-0.07	-0.15	0.03	-0.18	0.22	-0.24
Enzymes	αG	0.40	-0.54*	-0.30	0.51*	0.64**	0.30	0.69**	0.23	0.45	0.04	0.44
	βG	0.57*	-0.41	-0.40	0.67**	0.50*	0.38	0.42	0.16	0.37	-0.03	0.22
	βX	0.54*	-0.30	-0.40	0.64**	0.32	0.36	0.23	0.25	0.32	0.11	0.15
	NAG	0.30	-0.06	-0.49*	0.30	-0.46	-0.06	-0.52*	-0.38	-0.34	-0.38	-0.43
	AP	0.28	0.00	-0.16	0.09	-0.44	-0.21	-0.48*	-0.36	-0.38	-0.32	-0.33
	PPO	0.86**	-0.57*	-0.33	0.72**	0.25	-0.01	0.23	-0.13	0.05	-0.28	0.14
	PER	0.81**	-0.54*	-0.12	0.61**	0.37	-0.01	0.32	-0.03	0.13	-0.18	0.23

Values are *r* value of Pearson correlation analysis. * indicates a significant difference at $P < 0.05$; ** indicates a significant difference at $P < 0.01$. G^+ gram positive bacteria, G^- gram negative bacteria, *PLFAs* total PLFAs, G^+/G^- ratio of G^+ to G^-, *F/B* ratio of fungi to bacteria. *αG* α-1,4-glucosidase, *βG* β-1,4-glucosidase, *NAG* β-1,4-N-acetylglucosaminidase, *βX* β-1,4-xylosidase, *AP* acid phosphatase, *PPO* phenol oxidase, *PER* peroxidase. The same below

Table A3 Pearson correlation analysis of soil different PLFA contents and potential enzyme activities

Factors	G^+	G^-	Bacteria	Fungi	Actinobacterias	PLFAs	G^+/G^-	F/B
αG	0.29	0.46	0.53*	0.51*	0.61**	0.48*	0.12	-0.17
βG	0.67**	0.57*	0.83**	0.65**	0.70**	0.83**	0.52*	-0.27
βX	0.71**	0.46	0.73**	0.58*	0.47	0.73**	0.60**	-0.28
NAG	0.40	-0.15	0.01	0.02	-0.11	0.02	0.52*	-0.02
AP	0.32	-0.24	0.03	-0.14	-0.15	0.08	0.49*	-0.07
PPO	0.84**	0.09	0.57*	0.28	0.46	0.64**	0.91**	-0.44
PER	0.79**	0.04	0.55*	0.21	0.47*	0.62**	0.86**	-0.46

Table A4 Soil abiotic properties in different months

Treatment	Time	ST (℃)	SWC (%)	pH	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TN (g kg^{-1})	DOC (mg kg^{-1})	POC (g kg^{-1})	SOC (g kg^{-1})	POC/SOC (%)	SOC/TN
None	April	18.9±0.3aA	22.8±0.5aA	4.88±0.04aA	4.9±0.8aA	23.1±1.8bA	1.29±0.08aA	45.9±3.5bA	4.36±0.63aA	19.7±1.7aA	21.9±1.5aA	15.3±0.8aA
	July	28.1±0.2aA	18.8±0.5aB	4.80±0.04aA	6.5±0.4aA	14.6±0.4bB	1.13±0.06aA	40.5±3.6bA	3.03±0.37aA	16.9±0.7aA	18.1±2.2bA	15.4±0.9aA
	November	16.4±0.2aC	24.1±1.0bA	4.95±0.04aA	3.1±0.3aB	6.4±0.4aC	1.16±0.03aA	25.6±0.2bA	3.55±0.03bA	16.3±0.3bA	21.8±0.4aA	14.0±0.6aA
Understory	April	18.8±0.0aB	22.6±0.6aB	4.89±0.07aA	4.9±0.7aB	29.8±2.1aA	1.29±0.00aA	57.3±4.0aA	5.17±0.43aA	20.3±0.9aA	25.6±1.5aA	15.8±0.7aA
	July	27.6±0.2bA	19.9±0.4aC	4.86±0.07aA	7.1±0.4aA	29.24±0.8aA	1.29±0.03aA	51.4±5.0aA	4.48±0.84aA	19.9±1.2aA	22.1±2.9aA	15.4±0.7aA
	November	16.5±0.2aC	26.3±0.9aA	4.86±0.04aA	4.5±0.3aB	7.8±0.2aB	1.32±0.01aA	27.5±0.2aA	4.93±0.28aA	19.7±0.3aA	24.9±1.0aA	15.0±0.3aA

Different lowercase letters represented significant difference between different treatments, and different uppercase letters represented significant difference among different months in the same treatment ($P < 0.05$). The same below

Table A5 Soil PLFA contents in different months

Treatment	Time	G^+ (nmol g^{-1})	G^- (nmol g^{-1})	Bacteria (nmol g^{-1})	Fungi (nmol g^{-1})	AMF (nmol g^{-1})	Actinobacterias (nmol g^{-1})	PLFAs (nmol g^{-1})	G+/G-	F/B
None	April	4.25±0.44aB	4.61±0.50aA	8.86±0.94aA	2.07±0.30aA	0.36±0.05aB	2.10±0.22aA	11.56±0.75bA	0.93±0.01aB	0.21±0.01aAB
	July	6.28±0.47aA	3.62±0.08aAB	9.31±0.13bA	1.89±0.03bA	0.69±0.05aA	2.09±0.22aA	13.29±0.30aA	1.59±0.07aA	0.20±0.00aB
	November	2.82±0.34bB	3.11±0.22aB	5.93±0.56bB	1.45±0.07bA	0.35±0.04aB	0.817±0.41aB	8.19±0.52bB	0.90±0.05aB	0.25±0.02aA
Understory	April	3.81±0.46aC	4.32±0.21aA	10.53±0.54aA	2.21±0.08aA	0.43±0.26aB	2.05±0.06aAB	14.62±0.50aAB	0.89±0.05aB	0.26±0.04aA
	July	7.22±0.25aA	4.52±0.29aA	11.76±0.51aA	2.23±0.04aA	0.73±0.43aA	2.99±0.36aA	16.67±0.71aA	1.62±0.04aA	0.19±0.01aA
	November	5.41±0.51aB	4.92±0.28aA	10.32±0.59aA	2.35±0.21aA	0.47±0.45aB	1.23±0.55aB	13.90±0.98aB	1.13±0.15aB	0.23±0.03aA

667 Table A6 Soil potential enzyme activities in different months

Treatment	Time	αG (nmol g^{-1} h^{-1})	βG (nmol g^{-1} h^{-1})	βX (nmol g^{-1} h^{-1})	NAG (nmol g^{-1} h^{-1})	AP (nmol g^{-1} h^{-1})	PPO (nmol g^{-1} h^{-1})	PER (nmol g^{-1} h^{-1})
None	April	3.93±0.41aA	61.9±4.3aAB	24.8±0.2aB	24.9±3.2aA	300.5±22.9aB	0.18±0.02aB	0.40±0.03bB
	July	3.74±0.09aA	66.7±1.3aA	33.6±2.7aA	29.3±3.1bA	711.9±79.8aA	0.41±0.02aA	0.69±0.03bA
	November	2.48±0.12aB	52.8±2.1aB	30.5±1.7aAB	22.8±2.0bA	698.63±70.3aA	0.20±0.03aB	0.47±0.02aB
Understory	April	3.72±0.15aA	65.9±3.9aA	21.3±5.8aA	26.8±3.1aB	492.4±48.8aB	0.24±0.01aC	0.52±0.03aB
	July	3.35±0.19aAB	75.8±6.1aA	33.3±1.8aA	41.6±2.1aA	699.5±47.8aA	0.48±0.01aA	0.89±0.04aA
	November	2.90±0.12aB	65.7±2.3aA	33.8±2.8aA	32.6±1.6aB	689.32±35.1aA	0.28±0.01aB	0.53±0.04aB

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