Effects of Forest Age on Soil Fungal Community in a Northern Temperate Ecosystem

Han Zhiguang¹ · Sui Xin² · Li Mengsha²

Received: 18 December 2015 / Accepted: 24 March 2016 / Published online: 22 April 2016
© Association of Microbiologists of India 2016

Abstract The polymorphisms of soil fungal rDNA Internal Transcribed Spacer regions were studied in Korean pine forests of various ages (10–100-year-old trees) by means of cloned libraries, and analyzed to determine the effects of the trees' developmental stage on soil fungal community structure. The obtained Shannon diversity index (H) and richness (S) indicated that the diversity of the soil fungal community increased significantly with the development of Korean pines ($P < 0.05$). In addition, cluster analysis (UPGMA) showed that the soil fungal community variety associated with differently aged Korean pines was higher than 50 %. The soil fungal community diversity correlated significantly with the N content and C/N ratio of the soil ($P < 0.05$). The results of this study indicate that the age of in Korean pine can affect soil fungal community by altering soil properties, which in turn could affect the nutrient cycling in the forest ecosystem.

Keywords ITS · Soil DNA · PCR primers

Introduction

Soil fungi are an important component of forest ecosystems and have a broad range of functions in ecosystem processes [1]. The fungal community plays an important role in nutrient cycling through mycorrhizal associations and litter decomposition, has direct impacts on soil biochemical

processes, soil nutrient composition and conversion, and also strongly affects plant diversity via mutualistic or pathogenic interactions [2, 3]. Different soils contain a great diversity of fungal species, whose spatial distribution results from many factors, such as plant diversity, soil properties, land use and dispersal, and climate [4, 5]. Soil fungal community structures, in turn, directly affect the properties and fertility of soil, thereby influencing the growth of plants; the soil microbial population and abundance is thus positively correlated with soil fertility and plant growth status [6–8]. Hence, it has practical significance to investigate the distribution characteristics of soil microbiota, in which fungi take up an important position.

Changbai Mountains, situated in Jilin province, China, is one of the richest areas of temperate zone forests, and its native forests are an important source for wood in China. Its forest coverage is important to maintain the balance of this ecological environment. Previous research focused mainly on the restoration and protection of forest, but not on the soil, its contents and nutrients, ignoring possible soil microbial differences between forests of different ages [9, 10]. An understanding of the structure and composition of the soil microbial community of differently aged forests is essential, which we studied in the Changbai Mountain forest ecosystem.

In the past, research on soil micro-organisms was based on enrichment, separation and culture, however, only a fraction (estimated between 0.01 and 10 %) of the microorganisms present in the environment can be cultured by existing technology, which introduces bias in the diversity of the microorganisms under study.

Molecular methods based on Internal Transcribed Spacer (ITS) rDNA allow rapid detection of microbial diversity and have become essential in modern microbial ecology research, with the clone library technology as a

✉ Li Mengsha
lims99999@sina.com

¹ Tsinghua University, Beijing 100084, China

² Institute of Nature and Ecology, Heilongjiang Academy of Sciences, Harbin 150040, China

more advanced method of molecular ecology. The method is simple, rapid, has a high resolution and good repeatability, and produces quantitative results; with these advantages it has been widely used to study the diversity of a variety of microbial communities [11–13]. Since the application of molecular biological techniques overcomes the limitations of traditional methods based on culture, it provides a more accurate estimate of the richness and evenness on the level of genes and identifies variation in species, which provides a more objective insight in the community composition of fungi present in the environment.

Given that the age of a forest is one of the dominating factors determining the microbial community structure in soils, as well as the fact that our previous study showed that bacterial community compositions differ between different forest succession stages in Changbai mountains, we hypothesized that the soil fungal communities might change with different forest ages in this region [14–17]. In order to test this hypothesis, we collected soil samples from five native forests and estimated the composition, diversity and phylogeny of soil fungal communities in the samples by ITS rDNA clone library analysis.

Materials and Methods

Site Description and Sampling

Soil samples were collected in Changbai Mountains forest ecosystem, Jilin province in June 2011. Five Korean-pine broadleaf forest plots were selected with various ages of 10, 30, 50, 70 and 100 years, following determination of the local dominant tree population's ages (Table 1). Three cores with 10 cm depth of forest soil (starting from the surface) were mixed per plot, and a total of ten plots were collected and transported in sealed plastic bags. Organic C and total N were measured with the dichromate oxidation

method and Kjeldahl method [18], respectively. After addition of an equal volume of water, soil pH was measured. Nitrate in the form of ammonium (NH_4^+) and nitrate (NO_3^-) were measured by FLAstar 5000 analyzer (Foss Tecator AB Sweden Supply Company, Hoganas, Sweden).

DNA Extraction

After removal of visible root fragments, the soil samples were homogenized. Total soil DNA was extracted from 0.25 g fresh homogenated soil using the UltraCleanTM 12888 power soil extraction DNA kit (Mo Bio Laboratories Inc., USA). DNA was dissolved in 100 μL of pure water and used as template for PCR. The DNA samples were frozen at -80°C .

ITS rDNA Gene Amplification

By means of primers ITS1F (CTTGGTCATTGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) the ITSrDNA was amplified [14]. PCR reactions were performed in 50 μL including 2 μL of purified DNA (10–20 ng), 1.5 mM MgCl_2 , 0.2 mM dNTP, 0.5 mM of primers ITS1F and ITS4 and 2.5 U Taq DNA polymerase (TAKARA, Japan). The PCR reaction program included initial denaturation for 5 min and amplification for 34 cycles at 94°C for 60 s, 51°C for 60 s, 72°C for 60 s and a final extension at 72°C for 8 min. PCR products were purified using Tiangen PCR purification kit (Tiangen Biotech, P.R. China).

Cloning and Sequencing of PCR-Amplified Products

A total of fifteen purified PCR products, obtained from five forest types, were cloned using the PMD-19T vector system (TAKARA, Japan) according with the manufacturer's instructions. *Escherichia coli* DH5 α was used for transformation and clones were detected using the blue-white

Table 1 Description of the study sites in the Changbai Mountains, Northeast of China

Plot (age in years)	Latitude	Longitude	Altitude (m)	Tree species
Broad young mixed forest (10)	042 21'09.7"N	127 56'28.8"E	912	<i>Betula platyphylla</i> , <i>Larix gmelinii</i> , <i>Fraxinus mandshurica</i> , <i>Pinus koraiensis</i>
Broad mid-young forest (30)	042 24'03.1"N	128 05'54.1"E	763	<i>Betula platyphylla</i> , <i>Populus davidiana</i> , <i>Pinus koraiensis</i>
Broad mature mixed forest (50)	042 21'28.9"N	127 59'00.7"E	835	<i>Betula platyphylla</i> , <i>Populus davidiana</i> , <i>Pinus koraiensis</i>
Broad-leaved Korean pine mixed mature forest (70)	042 24'05.2"N	128 05'31.6"E	766	<i>Pinus koraiensis</i> , <i>Populus davidiana</i>
Broad-leaved Korean pine old forest (100)	042 21'04.6"N	127 59'15.8"E	833	<i>Pinus koraiensis</i>

screening method. Plasmids containing ITS rDNA gene sequences were extracted and purified using QIAGEN Plasmid spin miniprep Kit (Qiagen). A total of 100 white clones for each forest type were selected to sequence by M13 forward and reverse primer pairs in two directional sequencing with an ABI377 automatic sequencer (Applied Bio-systems, USA).

Sequence Analysis and Phylogenetic Tree Construction

The obtained ITS rDNA gene sequences were compared with sequences in GenBank using the basic local alignment search (BLAST) tool [19]. When a sequence reported more than one identical e-value, we selected the first sequence. After that, the ITS rDNA gene sequences were aligned by use of Clustal_X software and these alignments were used to calculate their distances by the two-parameter method [20, 21]. A phylogenetic tree was constructed using the neighbor-joining method [22]. Bootstrap analysis was performed based on 1000 re-samplings. All sequences were analyzed using MEGA4.0 software [23]. The obtained fungal ITS sequences have been deposited in GenBank with accession numbers JQ666320–JQ666842.

Results and Discussion

Soil Fungal Community Structure

We used the clone library technique to analyze the composition of fungal communities present in soil from different aged Korean pine forests, and found no significant difference in soil fungus community structures between the samples of plots covered with 30 year-old trees and those with 50 year-old trees (Fig. 1). The results of Shannon–Wiener index (H) and Richness index (S) showed that the variety of soil fungal community diversity and abundance

Table 2 Species diversity of the soil fungal microbiota

Forest ages	OTU	Chao	Ace	Shannon index	Simpson index
10	44	115	197	3.15	0.0803
30	44	127	334	3.14	0.0777
50	39	567	678	2.57	0.1964
70	53	120	273	3.48	0.0486
100	19	97	406	2.23	0.1356

first increased and then decreased with growth of the Korean pines, being lowest in soils of forests aged 10 and 100 years and highest for 50 years, followed by 70 and 30 years (Table 2).

Soil characteristics such as pH, ammonium nitrogen, nitrate nitrogen, total nitrogen (N) and carbon (C) content were also determined in the samples (Table 3). As can be seen, pH and ammonium nitrogen content followed similar trends, with an increase between 10 and 30 years and a downward trend from 30 to 100 years. In contrast, total nitrogen and carbon content decreased between 10 and 30 years to increase from 30 to 100 years. The content of nitrogen in the form of nitrate decreased first and then increased with the increase of the forest's age.

We searched for correlations between changes in soil fungal community diversity and soil chemical constituents, and found a significant positive correlation of soil fungal diversity and abundance with nitrate nitrogen and ammonium nitrogen ($P < 0.01$), and a significant negative correlation with carbon–nitrogen ratio ($P < 0.01$) (Table 4).

Analysis of Fungal Community Structure

Soil fungal community composition was inconsistent in the topsoil under the five different ages Korean pine forests. The soil communities of each plot consisted of 5 phyla, listed in decreasing of presence of representatives:

Fig. 1 Cluster analysis of soil fungal communities in different age stages of broad-leaved Korean pine forest

Table 3 Soil chemical properties of the different age stages

Forest ages	Nitrate nitrogen (mg/kg)	Ammonium nitrogen (mg/kg)	N (%)	C (%)	pH
10	14.63 ^a	4.13 ^c	0.68 ^a	9.63 ^{a,b}	4.63 ^d
30	13.04 ^a	8.56 ^a	0.60 ^a	8.16 ^b	5.53 ^a
50	6.32 ^a	2.79 ^d	0.71 ^a	9.54 ^{a,b}	5.10 ^b
70	14.38 ^a	4.53 ^{b,c}	0.75 ^a	9.42 ^{a,b}	5.20 ^a
100	16.09 ^a	5.46 ^b	0.92 ^b	11.78 ^a	4.67 ^c

^{a–d} Different letters in the same column means significant differences at 0.05 levels among samples

Table 4 Pearson's correlation analyses between the fungal community's diversity and soil properties from all sites

	Nitrate nitrogen (mg/kg)	Ammonium nitrogen (mg/kg)	N (%)	C (%)	C/N	pH
F–H	0.540**	0.374	0.354	−0.034	−0.596**	−0.378
F–S	0.643**	0.323	0.396	−0.041	−0.653**	−0.218

F–H: diversity of fungal ITS sequences; F–S: richness of fungal ITS sequences

** Significant difference at 0.01 levels among samples

Fig. 2 The fungi composition of the analysed samples of the five Korean pine forest ages

Basidiomycota > *Ascomycota* > *Zygomycota* > *Fungiunclassified* > *Chytridiomycota*. Among these phyla, *Basidiomycota*, *Ascomycota*, *Zygomycota*, and unclassified *Fungi* dominated the fungal communities, accounting for 50.1, 22.9, 13.3 and 12.1 % of the total fungi, respectively. Other phyla were considered minority groups, accounting for only 0.58 % of the total fungal community.

The abundance of the 5 phyla varied with changes in forest age. Patterns of abundance in the fungal community showed inconsistent variation between forest ages. For example, the abundance of *Ascomycota* was highest in the 10 years old sample but lower in the 70 years old sample, while lower still in the 50, 30 and 100 years samples. In contrast, *Basidiomycota* exhibited the

opposite pattern. The phyla distribution also varied inconsistently with variation in nitrogen content. For example, the abundance of *Zygomycota* decreased first and then increased with increased forest ages, whereas unclassified *Fungi* decreased. Forest age had a great impact on fungal abundance (Fig. 2).

The Soil Fungal Community Structure Similarity of Different Forest Age Stages

Analysis of different age stages with soil fungal community structure resulted in a similarity coefficient that showed a gradual decline from 10 years onwards (Table 5). This shows that soil fungal community structure and function changed over time.

Table 5 Similarity coefficient of soil fungal community in different forest age stages

Forest ages	10	30	50	70	100
10	1				
30	0.307	1			
50	0.248	0.245	1		
70	0.211	0.183	0.211	1	
100	0.194	0.212	0.205	0.258	1

Soil microorganisms are an important part of soil ecosystems and play an important role in maintaining a stable and healthy environment for tree development. In this study, we determined changes in fungal community structure as reported by the fingerprints of fungal ITS sequences, and used the diversity index (H, S) to evaluate the soil fungal community diversity, as reported by the diversity indices obtained [3]. The present study demonstrates that the diversity and abundance of soil fungal community from different ages of Korean pine forest first increased and then decreased with Korean pine growth, probably reflecting the difference of vegetation type and age [21, 24].

Moreover, we identified trends in the composition of fungal microbiota with increasing forest age (Fig. 2). For instance, the abundance of *Basidiomycota* increased, while *Ascomycota* decreased from 10 to 100 years. This maybe related to increased litters and its components, since *Basidiomycota* is mainly responsible for decomposition of lignin. In a young forest, trees are in a rapid growth stage, which results in few litters containing little lignin, and this could explain why *Basidiomycota* is less abundant here. As the forest grows, it becomes more complex and litters contain more lignin, allowing growth of *Basidiomycota*. Likewise, *Zygomycota* increased when 10 years old forests were compared to 70 years old forests, but was decreased in 100 years old forests. Such trends have been observed before, for example, Liu found that soil microbial community structure changed with the *Cunninghamia lanceolata* plantation's development stage [25].

A young forest of Korean pines has less litter resulting in a low diversity of the microbial community. With the growth of Korean pines, soil nutrition improves together with an increase in the soil microbial diversity, to reach a maximum in 70 years.

By correlation analysis, we found that community diversity and richness of different forest stages correlated with abundance of soil nitrogen. Soil nitrogen content can be the limiting factor of biological growth; many studies have shown that soil nitrogen content can influence soil microbial biomass, activity and community composition [22]. Presence and abundance of fungi is more important

than other microorganisms in the surface of soil, while bacteria are more important for degradation of the buried organic matter [23]. That study also found that soil fungal diversity positively correlated with soil ammonium nitrogen. Soil fungi were positively correlated with available N, and possibly correlated with microbial functional groups that involved in nitrogen turnover [26, 27]. Further research on soil microbial functional groups is required to explore the interaction between soil microorganism and soil nutrient dynamics.

Fungi have a direct impact on soil organic matter content through decomposing of organic material, whereas the amount of soil organic matter can also change soil quantity and composition of soil fungi [28]. Soil C to N ratio (C/N) is another important factor that influences soil microbial community structure [29]. Correlation analysis presented here showed that the fungal diversity correlated significantly in a negative manner with C/N, which was consistent with Bazzaz and Williams [30], who reported that microbial populations are affected by soil C/N so that when the soil organic matter content increased, the soil microbial diversity decreased. Wang et al. [31] also found that fungal structure community negatively correlated with C/N, presumably because a high concentration of nitrogen inhibits growth of fungi. Our study confirmed that soil fungal diversity negatively correlated with soil C/N, indicating that high soil organic matter C/N didn't support optimal microorganism growth and slowed down the soil organic matter turnover. Luo [32] used the DGGE method to study soil microbial communities of long-term fertilized soils, reporting that fertilization changed both soil C/N and microorganism community structure, and both had a significant correlation. Wan [33] used the PFLA method to study the relationship of soil microorganism community and soil pH and C/N in a Swedish boreal forest, and found that changes in soil C/N significantly affected the soil microbial community structure, thereby affecting the nitrogen supply to plants. Finally, Ushio et al. [34] reported that the C/N and soil microbial community structure were different in coniferous and broadleaf forests and that the types of returned soil organic matter could cause changes in soil properties and microbial community composition.

Conclusion

In this study, we discovered that the diversity and abundance of soil fungal community increased first and then decreased as Korean Pine forest matures, and we revealed relationships between soil fungal communities and soil available N and C to N ratios. These findings imply that soil microorganism community composition changes with the physical and chemical properties of the soil, which

again affects particular functional group composition and nutrient cycling within the forest ecosystem. This would eventually result in a productivity decline over time in Korean Pinenative forests. The results provide important information on forest ecology in general and for in Changbai Mountain forest in particular, and provides a scientific basis for further evaluation of the soil conditions from a microbiological point of view.

Acknowledgments This study was supported by National Natural Science Foundation (31470019).

References

- Tardy V, Chabbi A, Charrier X, de Berranger C, Reigner T, Dequiedt S, Faivre-Primot C, Terrat S, Ranjard L, Maron PA (2015) Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. *PLoS One* 10:e0130672. doi:10.1371/journal.pone.0130672
- Haňáčková Z, Koukol O, Štursová M, Kolařík M, Baldrian P (2015) Fungal succession in the needle litter of a montane *Picea abies* forest investigated through strain isolation and molecular finger printing. *Fungal Ecol* 13:157–166. doi:10.1016/j.funeco.2014.09.007
- Urbanová M, Šnajdr J, Baldrian P (2015) Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biol Biochem* 84:53–64. doi:10.1016/j.soilbio.2015.02.011
- Rillig MC, Aguilar-Trigueros CA, Joana B, Erik V, Veresoglou SD, Anika L (2015) Plant root and mycorrhizal fungal traits for understanding soil aggregation. *New Phytol* 205:1385–1388. doi:10.1111/nph.13045
- Liang Y, He X, Chen C, Feng S, Liu L, Chen X, Su Y (2015) Influence of plant communities and soil properties during natural vegetation restoration on arbuscular mycorrhizal fungal communities in a karst region. *Ecol Eng* 82:57–65. doi:10.1016/j.ecoleng.2015.04.089
- Long RJ, Dong SK, Wei XH, Pu XP (2005) The effect of supplementary feeds on the bodyweight of yaks in cold season. *Livest Prod Sci* 93:197–204. doi:10.1016/j.livprodsci.2004.08.016
- Wang QK, Wang SL (2008) Soil microbial properties and nutrients in pure and mixed Chinese fir plantations. *J For Res* 19:131–135. doi:10.1007/s11676-008-0022-7
- Cozzolino V, Di Meo V, Monda H, Spaccini R, Piccolo A (2016) The molecular characteristics of compost affect plant growth, arbuscular mycorrhizal fungi, and soil microbial community composition. *Biol Fertil Soils* 52:15–29. doi:10.1007/s00374-015-1046-8
- Yao J, He X, He H, Chen W, Dai L, Lewis BJ, Yu L (2016) The long-term effects of planting and harvesting on secondary forest dynamics under climate change in northeastern China. *Sci Rep* 6:18490. doi:10.1038/srep18490
- Yu DY, Shi PJ, Han GY, Zhu WQ, Du SQ, Xun B (2011) Forest ecosystem restoration due to a national conservation plan in China. *Ecol Eng* 37:1387–1397. doi:10.1016/j.ecoleng.2011.03.011
- Lukow T, Dunfield PF, Liesack W (2000) Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS Microbiol Ecol* 32:241–247. doi:10.1016/S0168-6496(00)00033-7
- Christian W, Bettina A, Christian G, Meckenstock RU, Tillmann L (2008) Depth-resolved quantification of anaerobic toluene degraders and aquifer microbial community patterns in distinct redox zones of a tar oil contaminant plume. *Appl Environ Microbiol* 74:792–801. doi:10.1128/AEM.01951-07
- Mummey DL, Stahl PD, Buyer JS (2002) Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. *Appl Soil Ecol* 21:251–259. doi:10.1016/S0929-1393(02)00090-2
- Rubinoff H, Fireman BH (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Mol Ecol* 11:2669–2678. doi:10.1046/j.1365-294X.2002.01647.x
- Bonet JA, Fischer CR, Colinas C (2004) The relationship between forest age and aspect on the production of sporocarps of ectomycorrhizal fungi in *Pinus sylvestris* forests of the central pyrenees. *For Ecol Manag* 203:157–175. doi:10.1016/j.foreco.2004.07.063
- Parádi I, Baar J (2006) Mycorrhizal fungal diversity in willow forests of different age along the river Waal, The Netherlands. *For Ecol Manag* 237:366–372. doi:10.1016/j.foreco.2006.09.059
- Sui X, Feng FJ, Lou X, Zheng JQ, Han SJ, Dai GH (2013) Effects of birch forest at different developmental stages on soil microorganism community structure. *J Bionosci* 7:78–83. doi:10.1166/jbns.2013.1085
- Jackson MLR, Barak P (2005) Soil chemical analysis: advanced course. UW-Madison Libraries Parallel Press, Madison
- Ann PJ, Huang JH, Tsai JN, Ko WH (2016) Morphological, molecular and pathological characterization of *Phytophthora amaranthi* sp. nov. from Amaranth in Taiwan. *J Phytopathol* 164:94–101. doi:10.1111/jph.12433
- Grayston SJ, Griffith GS, Mawdsley JL, Campbell CD, Bardgett RD (2001) Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biol Biochem* 33:533–551. doi:10.1016/S0038-0717(00)00194-2
- Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol Biochem* 33:1437–1445. doi:10.1016/S0038-0717(01)00052-9
- Sarathchandra SU, Ghani A, Yeates GW, Burch G, Cox NR (2001) Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. *Soil Biol Biochem* 33:953–964. doi:10.1016/S0038-0717(00)00245-5
- Li G, Liu Y, Li R, Guo B, Xu Y (2009) Responses of decomposition rate, nutrient return, and composition of leaf litter to thinning intensities in a *Pinus tabulaeformis* plantation. *Front Fore China* 4:458–463. doi:10.1007/s11461-009-0058-7
- Grayston SJ, Griffith GS, Mawdsley JL, Campbell CD, Bardgett RD (2001) Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biol Biochem* 33:533–551. doi:10.1016/S0038-0717(00)00194-2
- Liu L, Duan ZH, Wang SL, Hu JC, Hu ZG, Zhang QR, Wang SJ (2009) Effects of *Cunninghamia lanceolata* plantations at different developmental stages on soil microbial community structure. *Chin J Ecol* 28:2417–2423
- Bhaganagare GR, Kesawat MS, Das BK, Suresh A, Surwase BS, Manorama (2013) Assessment of nifH diversity in rhizobial isolates of different origin and the role of antioxidant in respiratory protection. *J Crop Sci Biotechnol* 16:17–22. doi:10.1007/s12892-012-0010-5
- Cavagnaro TR, Jackson LE, Scow KM, Hristova KR (2007) Effects of arbuscular mycorrhizas on ammonia oxidizing bacteria in an organic farm soil. *Microb Ecol* 54:618–626. doi:10.1007/s00248-007-9212-7
- Billings SA, Ziegler SE (2005) Linking microbial activity and soil organic matter transformations in forest soils under elevated

- CO₂. *Glob Change Biol* 11:203–212. doi:[10.1111/j.1365-2486.2005.00909.x](https://doi.org/10.1111/j.1365-2486.2005.00909.x)
29. Park KC, Kremer RJ (2010) Establishment of an inoculated bacterial strain on plant root surfaces in soils with different microbial community. *J Korean Soc Appl Biol Chem* 53:379–383. doi:[10.3839/jksabc.2010.059](https://doi.org/10.3839/jksabc.2010.059)
30. Bazzaz FA, Williams WE (1991) Atmospheric CO₂ concentrations within a mixed forest: implications for seedling growth. *Ecology* 72:12–16. doi:[10.2307/1938896](https://doi.org/10.2307/1938896)
31. Wang D, Ma FY, Yao XF, Xin H, Song X, Zhang ZX (2012) Properties of soil microbes, nutrients and soil enzyme activities and their relationship in a degraded wetland of Yellow River Delta. *Sci Soil Water Conserv* 10:94–98
32. Luo P, Han X, Yan W, Mei H, Hui S, Ning L (2014) Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. *Ann Microbiol* 65:1–10. doi:[10.1007/s13213-014-0889-9](https://doi.org/10.1007/s13213-014-0889-9)
33. Wan X, Huang Z, He Z, Yu Z, Wang M, Davis MR (2014) Soil c:n ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. *Plant Soil* 387:103–116. doi:[10.1007/s11104-014-2277-4](https://doi.org/10.1007/s11104-014-2277-4)
34. Ushio M, Wagai R, Balser TC, Kitayama K (2008) Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? *Soil Biol Biochem* 40:2699–2702. doi:[10.1016/j.soilbio.2008.06.023](https://doi.org/10.1016/j.soilbio.2008.06.023)