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REGULAR ARTICLE

Positive correlation between soil bacterial metabolic and plant species diversity and bacterial and fungal diversity in a vegetation succession on Karst

Xun-Yang He · Ke-Lin Wang · Wei Zhang · Zhi-Hui Chen · Yong-Guan Zhu · Hong-Song Chen

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Abstract The hypothesis that positive links exist among plant taxonomic diversity, belowground microbial taxonomic and metabolic diversities was tested for four secondary vegetation successional stages (tussock (T), shrub (S), secondary forest (SF) and primary forest (PF)) in Huanjiang county, SW China. Soil bacterial communities were characterized by DNA fingerprinting and metabolic profiling. Along the succession, Shannon diversity indices followed the order SF>PF>S>T for plant taxonomic diversity, T>SF>PF>S for bacterial operational taxonomic diversity, SF>T>S>PF for fungal operational taxonomic diversity, and SF>PF>S>T for bacterial metabolic diversity. Significant positive correlations were found between bacterial and fungal taxonomic diversities. However, there was no significant correlation between soil microbial taxonomic diversity and bacterial metabolic diversity. Two-way ANOVA revealed that vegetation and season, as well as their interaction, had significant effects on soil microbial (fungal and bacterial) taxonomic diversities, but that there were no seasonal effects on metabolic diversity. However, PCA and MANOVA revealed highly significant differences among the bacterial community-level physiological profiles, reflecting the successional sequence. The findings from this survey support the notion that there are strong interactions between aboveground and belowground communities and suggest that bacterial metabolic and plant taxonomic diversities, but not microbial taxonomic and metabolic diversities, can be correlated.

Keywords BIOLOG · Microbial community · PCR-DGGE · Seasonal variation · Vegetation effects

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X.-Y. He · K.-L. Wang (✉) · W. Zhang · Z.-H. Chen · H.-S. Chen
Institute of Subtropical Agriculture,
Chinese Academy of Sciences,
Changsha 410125, China
e-mail: kelin@isa.ac.cn

X.-Y. He
Graduate School of Chinese Academy of Science,
Beijing 100039, China

Y.-G. Zhu
Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences,
Beijing 100085, China

Abbreviations

CLPP	Community-level physiological profiling pattern
DGGE	Denaturing gradient gel electrophoresis
EDTA	Ethylene diamine tetraacetic acid
MANOVA	Multivariate analysis of variance
PCA	Principle component analysis
PC	Principle component
PCR	polymerase chain reaction
UPGMA	Unweighted pair group method with arithmetic mean

Introduction

Soil ecosystems are highly complex, containing a tremendous amount of species. Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning due to their capacity to determine nutrient cycles (Doran and Zeiss 2000). Land use change represents the most substantial human alteration of ecosystems, dramatically altering plant community compositions and typically lowering their diversity (Vitousek et al. 1997). The direct effects of changes in plant community composition and/or diversity on ecosystem processes have been evaluated in a number of studies (Naeem and Li 1997; Tilman 1999), as have their effects on soil microbial diversity (Bossio et al. 2005; Crecchio et al. 2007; Lupway et al. 1998; Yao et al. 2006). The relationship between aboveground plant diversity and belowground microbial diversity has also been examined: Carney and Matson (2006) studied the influence of tropical plant diversity on soil microbial communities within an experimental system, Costa et al. (2006) studied the effects of different crops on bacterial and fungal rhizosphere communities using molecular fingerprinting, and De Deyn and Van der Putten (2005) reviewed the link between aboveground and belowground diversity. However, little is known about how soil microbes respond to changes of plant diversity in secondary vegetation succession.

Karst is a distinctive topography, created by the action of acidic water on carbonate bedrock, such as limestone, dolomite, or marble. Globally, the Karst landscape occupies 22,000,000 km^2, accounting for 15% of the total land area (Yuan 1991). The Karst area of SW China covers 550,000 km^2 (Li et al. 2002), but Karst also occurs at the European Mediterranean seacoast and the east coast of South America (Ouyang 1998). An increasing human population and other heavy anthropogenic impacts have seriously damaged the vegetation in the Karst region of SW China (Yao et al. 2001). Proper ecosystem functioning is seriously threatened by soil degradation, which impairs water and nutrient flows, and ultimately plant growth (Pan and Cao 1999). Microbial communities are an important component of soil quality and may serve as indicators for changes in soil health (Scow et al. 1998), but the effects of ecosystem degradation on soil microbial communities are largely unknown.

In this study, involving investigations on microbial diversity at two seasonal time points along a vegetation succession, we addressed the question of whether positive links exist among plant taxonomic diversity, belowground microbial taxonomic and metabolic diversities; Moreover, seasonal variations on soil microbial taxonomic diversity were discussed.

Materials and methods

Study sites

Four secondary vegetation succession stages were selected around the Guzhou village, Huanjiang county, Guangxi province in SW China (24°50′ N, 107°55′ E). A subtropical mountainous monsoon climate dominates in the area with a mean annual rainfall of 1389 mm and a mean annual air temperature of 18.5°C. The wet season with 70% of annual precipitation lasts from April until end of August. The local Karst formation consists of layers of limestone, arranged horizontally with well developed vertical fissures. The shallow soils with depth of only 0–20 cm have been mollic inceptisols, but are largely eroded or degraded due to overuse, as is vegetation cover.

Three secondary successional communities (tussock (T), shrub (S), and secondary forest (SF)) were identified at the study site. The T community, in an area not cultivated for 3 years but suffering from substantial anthropogenic disturbance (grazing and mowing), is located in a peak-cluster depression at lower altitude and with less steep slopes than the others studied communities located in sharp hillsides. The S and SF communities had only experienced little occasional disturbance for 18 years and 50 years, respectively, due to the implementation of conservation projects in the area. A neighboring undisturbed primary forest community (PF) (25°07′54″ N, 108°00′08″ E) in the Mulun National Nature Reserve was selected as a control ecosystem characterized by different dominant species and undisturbed by human action. For each of the stages S, SF, and PF a single sampling site (with dominant species typical of the study region) was selected. For the T successional stage three sampling sites were chosen to account for the larger variability in plant cover observed in this initial stage of succession. The plots for plant survey

and soil sampling were 5 m×5 m for the T, 20 m× 10 m for the S, and 40 m×20 m for both the SF and PF vegetation successional stages.

Plant survey and soil sampling

Each plot (except for T) was subdivided into four rectangular subplots from which three were chosen for vegetation surveys in June 2006. In the SF and PF communities, each subplot (quadrat) was divided into three layers (arboreal, shrubby and herbaceous), whereas the vegetation in the S community subplots was divided into two layers (shrubby and herbaceous). For the arboreal layers, all woody stems (including climbing plants) with diameters at breast height (DBH) ≥2.5 cm were tallied, identified and measured for DBH to the nearest 0.1 cm. For the shrubby (tree seedlings and woody climbing plants <2.5 cm stem diameter) and herbaceous (herbaceous climbing plants and ferns) layers the fascicles and heights of each plant species were tallied. All plant species were identified and their percent cover was visually estimated.

From each plot (T) or subplot (S, SF, PF), five to eight soil sub-samples to a depth of 15 cm were collected along an S-shaped transect, using a soil corer 3 cm in diameter. The soil sub-samples were pooled to one composite sample per plot (T) or subplot (S, SF, PF), according to the method of Klose et al. (2004), yielding three replicates per successional stage. Sampling was carried out in June and December 2006, corresponding to the wet and dry seasons, respectively. The soil samples were stored in plastic bags in an icebox for transport to the laboratory, where they were kept at 4°C until analyzed. Sieved soil samples (<2 mm) were divided into three portions. One part of soil was stored at −70°C for PCR-DGGE fingerprinting, another part was stored at 4°C for BIOLOG profile analyses, and a further part was air dried for physical and chemical analyses. Soil properties are reported in Table 1.

DNA extraction and community fingerprinting

Total bacterial community DNA was extracted from 500 mg fresh soil, using the commercial FastDNA Spin Kit for Soil (BIO101, Vista, CA, USA) according to the manufacturer's protocol. Prior to PCR, the DNA was further purified with the GeneClean kit (BIO101). PCR was carried out with a PTC-100 thermal cycler (M.J. Research, Inc., Watertown, MA), and the primer systems targeted the small subunit rRNA genes of bacteria and fungi. For bacteria, amplification of 16S rDNA sequences was performed with the primer pair F984GC/R1378, yielding a 433 bp DNA fragment suitable for total community fingerprinting (Heuer et al. 1999). For fungi, the NS1 primer and the fungus-specific primer GCfung (May et al. 2001) were used to amplify the 5′ end of the 18S rDNA (370 bp). Both F984GC and GCfung had GC clamps of 40 bp DNA[1] (Muyzer et al. 1993), as needed for optimal DGGE analysis (Ferris et al. 1996). All PCR reactions were carried out in 50 μl with 0.02% (w/v) bovine serum albumin and 200 nM of each primer for bacteria, and 400 nM for fungi, 200 μM dNTPs (QIAGEN Inc., Hilden, Germany), 1.5U of HotStarTaq DNA polymerase (QIAGEN Inc., Hilden, Germany), and 1 μl (bacteria) or 4 μl (fungi) of 1:9 diluted template DNA (5~30 ng μl^{-1}). For bacteria the touchdown PCR protocol of Gelsomino and Cacco (2006) was used with initial denaturation at 95°C for 15 min. For fungi the initial denaturation (95°C for 15 min), was followed by 35 amplification cycles (95°C for 1 min, 57°C for 1 min, 72°C for 2 min), and a final extension at 68°C for 10 min, and cooling to 4°C.

DGGE was performed using a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). 20 μl of PCR products were loaded on 6% (bacteria) or 8% (fungi) wt/vol polyacrylamide gels in 1×TAE buffer (40 mM Tris base, 20 mM acetic acid, and 1 mM disodium EDTA, pH 8.3) containing a linear chemical gradient of 40–60% (bacteria) or 15–35% (fungi) denaturant (100% denaturant corresponding to 7 M urea in 40% (v/v) of deionized formamide). Electrophoreses were run in 1×TAE buffer at 60°C with a constant voltage of 100 V for 7 h. Images of gels stained with ethidium bromide were recorded with a Polaroid camera system (SYNGENE Inc., USA).

Bacterial community metabolic (BIOLOG) profiles

Community-level physiological profiling patterns of soil bacterial communities (CLPPs) were assessed

[1] GC-clamp sequence: 5′-CGCCCGGGGCGCGCCCCGGGC GGGGCGGGGGCACGGGGGG-′

Table 1 Soil parameters in the secondary vegetation succession stages tussock (T), shrub (S), secondary forest (SF), and primary forest (PF)[a]

Variables	*T*	*S*	SF	PF
pH (1:2.5 H_2O)	6.82±0.10a	7.72±0.02b	7.24±0.38ab	7.99±0.02b
SOM (g kg^{-1})	22.73±2.23a	59.9±4.00b	97.48±6.84c	90.04±10.69c
Total N (g kg^{-1})	1.16±0.15a	3.53±0.15b	5.68±0.4c	4.82±0.49c
C/N	11.51±0.49b	9.81±0.24a	9.95±0.19a	10.79±0.22ab
CEC ($cmol_{(+)}$ kg^{-1})	9.88±2.25a	28.05±0.55b	34.62±1.95b	32.56±3.13b
Sand (2–0.05 mm) (%)	17.38±4.64c	8.21±0.97ab	15.54±1.21cb	3.83±1.16a
Silt (0.05–0.002 mm) (%)	53.39±1.62c	38.43±0.62b	33.93±3.03a	30.16±2.34a
Clay <0.002 mm (%)	28.35±4.76a	51.2±0.64b	47.88±1.68b	45.03±3.47b
Bulk density (kg dm^{-3})	1.35±0.03c	1.17±0.03b	0.88±0.03a	0.86±0.07a

[a] Sampled in June 2006. Values are the means of three replicates with associated standard errors. Means in a row with the same letter are not significantly different at $P<0.05$ (Duncan)

using BIOLOG® 96-well Eco-Microplates (Biolog Inc., Hayward, USA) with 31 different carbon sources and a negative control (water), replicated three times in each microplate. Microorganisms were extracted from the soil samples according to Zak et al. (1994). 150 µl of a 1:1000 (w/v) soil suspension were dispensed into each of the 96 wells and then the microplates were incubated at 25°C in the dark for 7 days. Color development (reflecting carbon utilization) in the wells was followed by absorbance measurements at 590 nm every 24 h using a Microplate E-Max Reader (Bio-Rad).

Data analysis

Banding patterns of DGGE profiles were analyzed with the Bio-Rad Quantity One software to calculate Dice distances among lanes and draw UPGMA similarity dendrograms (Crecchio et al. 2004). After subtracting the background from the gradient gel image by the strip method, lane background subtraction of the nonlinear background was achieved by using the rolling disk mechanism with an intensity of 5. For identification and quantification of the DNA bands the tolerance and optimization parameters were set to 1%. To eliminate variation in band intensity, caused by different amounts of loaded PCR product relative ribotype intensities per lane were calculated by dividing the intensity of the corresponding band by the sum of the intensities of all bands within the lane.

In the analysis of the BIOLOG data the midpoint (72-h incubation) in overall color development was used as a reference point of color development changes with time (Garland 1996). The number of positive wells were counted according to Glimm et al. (1997), with an OD correction to 2 when OD>2 as these positive wells could contribute to the metabolic diversity. The CLPP data were analyzed by PCA after accounting for the different initial cell densities (Garland 1997) and logarithmic transformation (Weber et al. 2007). Principal components were calculated on the basis of the covariance matrix, since all variables were based on the same scale and may therefore contain information (Weber et al. 2007). MANOVA was conducted for statistical comparisons of community catabolic profiles among treatments (Glimm et al. 1997).

For the plant, DGGE, and utilization data, Shannon indices were calculated as $H' = -\sum (p_i)(\ln p_i)$ (Magurran 2004), where p_i is (a) the importance value of the *i*th plant species, which was calculated as the sum of the relative density, relative abundance and relative frequency for arboreal layer, and as the sum of the relative height and relative cover for shrubby and herbaceous layer in each plot, respectively (Ou et al. 2005), (b) the percentage of the total intensity accounted for by the i^{th} band (Müller et al. 2002), or (c) the total coloration accounted for by the i^{th} substrate (Zak et al. 1994), respectively. Here, Shannon diversity indices were chosen, because they do not only reflect richness, but also evenness of communities (Magurran 2004). Shannon diversities were compared by two-way analysis of variance (ANOVA), using SPSS11.5 (SPSS Inc., Chicago, USA) software. Vegetation and sampling time were used as fixed factors. Comparisons among successional stages were made using Duncan's test at $P<0.05$.

Results

Plant survey

The three T communities had different dominant species (Table 2). The shrubby layer of the S and the arboreal layer of the SF communities shared the same dominant evergreen broadleaved species (i.e. *Bauhinia brachycarpa var.cavaleriei*), which was one of prevalent evergreen broadleaved species in the study area. The SF and PF plant communities belong to the evergreen broadleaved forest and evergreen-deciduous broadleaved mixed forest, respectively. The tree layer of the SF community was dominated by the evergreen broadleaved species (*Bauhinia brachycarpa var.cavaleriei, Sterculia euosma, Phoebe calcarea*; Table 2). A completely different set of species, including two deciduous arboreal trees (*Platycarya longipes* and *Boniodendron minius*) and one evergreen arboreal tree (*Rapanea neriifolia*), were dominant in PF (Table 2). Plant species diversity in the four succession stages followed the order: SF>PF>S>T, with significant differences between the T, S, and SF communities (Fig. 4a).

Table 2 The dominant plant species for a vegetation succession on Karst [a]

Stages	Layer	Ranking of dominant plant species
T1	H	*Imperata cylindrica* (0.27)
		Selaginella delicatula (0.21)
		Ficus tikoua (0.19)
T2	H	*Microstegium vegans* (0.29)
		Erigeron acer (0.23)
		Neyraudia reynaudiana (0.2)
T3	H	*Apluda mutica* (0.52)
		Lysimachia alfredii var. alfredii (0.13)
		Sanicula chinensis (0.11)
S	S	*Bauhinia brachycarpa var.cavaleriei* (0.37)
		Broussonetia papyrifera (0.13)
		Alchornea trewioides (0.1)
	H	*Pueraria lobata* (0.27)
		Imperata cylindrica (0.19)
		Paederia scandens var. tomentosa (0.08)
SF	A	*Bauhinia brachycarpa var.cavaleriei* (0.24)
		Sterculia euosma (0.15)
		Phoebe calcarea (0.14)
	S	*Bauhinia championii var. championii* (0.17)
		Dalbergia hancei (0.1)
		Lonicera japonica (0.06)
	H	*Nephrolepis auriculata* (0.36)
		Neyraudia reynaudiana (0.13)
		Drynaria roosii (0.1)
PF	A	*Platycarya longipes* (0.21)
		Boniodendron minius (0.21)
		Rapanea neriifolia (0.19)
	S	*Ampelocalamus calcareous* (0.16)
		Rapanea neriifolia (0.12)
		Murraya euchrestifolia (0.09)
	H	*Carex sp.* (0.37)
		Clematis florida (0.16)
		Cymbidium cyperifolium (0.07)

[a] H, S and A refer to the herbaceous, shrubby and arboreal layer, respectively. Value in parentheses indicates the important value of plant species (see associated section in Materials and methods). For succession stage abbreviations see Table 1

Microbial taxonomic diversity

Variability among sample replicates in the composition of the bacterial communities was considerable (Fig. 1a). At the summer and winter sampling times the bacterial communities from PF soils were distinct from those of the other soils, with low variability among replicates (59–76% similarity). Conversely, the communities in the S and T soils showed more variability, ranging between 42–86% and 42–66% similarity, respectively. Seasonal community differences were bigger than those among sample replicates (Fig. 2a). In contrast to the bacterial communities the fungal communities (Fig. 1b) showed even more variability, but also more distinctly dominant ribotypes. Variability was highest among replicates of the PF succession stage (12–50% similarity, Fig. 2b) with the consequence that these fungal communities could not be distinguished from the other ones.

Bacterial community metabolic (BIOLOG) diversity

The catabolic capability of soil bacterial communities differed considerably among successional stages. PCA on bacterial CLPPs showed that PC1 accounted for 28.1% and PC2 for 17.7% of the total variance (Fig. 3). A multivariate analysis (Wilkinson's lambda) on CLPP differences was conducted, using the first five PCs which account for 75.1% of total variation. First, the comparisons among four treatments resulted in an F-criterion of 7.19 at 35, 53 degrees of freedom ($p<0.001$), indicating significant differences of CLPPs among the bacterial communities. Subsequent

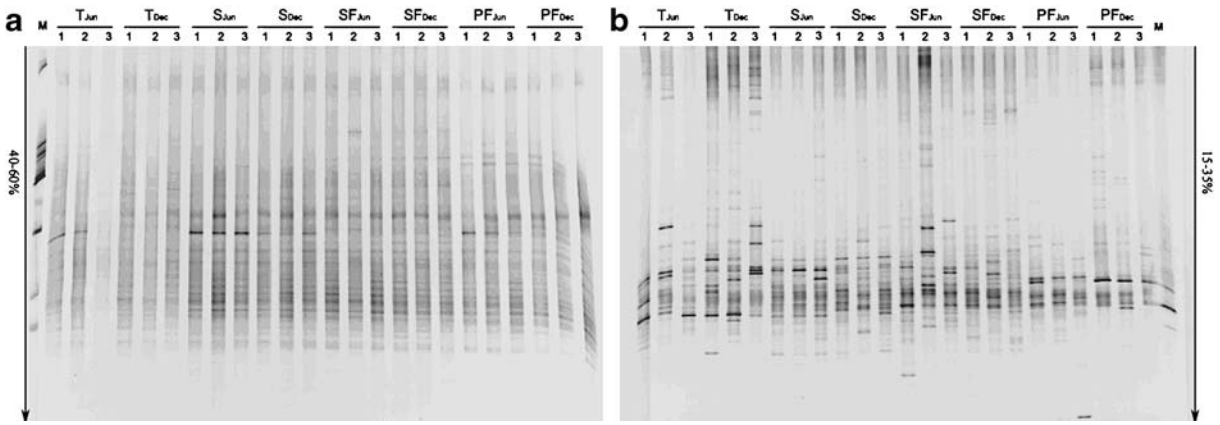

Fig. 1 DGGE community fingerprints of PCR amplified bacterial 16S (**a**) and fungal 18S (**b**) rDNA fragments from secondary vegetation succession stages. Capital letters denote vegetation successional stages (*T* tussock, *S* shrub, *SF* secondary forest, *PF* primary forest) and subscripts months of sampling. Numbers stand for replicate soil samples. *M* refers to the 100 bp DNA ladder for bacteria and a collection of previously detected fungi, respectively

Univariate tests for different PCs indicated that three groups (T, SJun and the others) showed significant differences ($p<0.05$) in Post Hoc Tests on PC1 (Fig. 3).

Differences in microbial taxonomic and metabolic diversity

Vegetation and season, as well as their interaction, had significant effects on bacterial taxonomic diversity ($p<0.05$, $p<0.001$, $p<0.05$, respectively), as well as fungal taxonomic diversity ($p<0.001$, $p<0.001$, $p<0.01$, respectively); vegetation and the interaction of vegetation and season ($p<0.001$, $p<0.05$, respectively), but not season, had significant effects on bacterial metabolic diversity (Table 3).

Multiple comparison (Duncan test) of Shannon diversity indices indicated that the metabolic diversity of bacterial communities in SF were significantly different ($p<0.05$) from those of the other succession stages in the same season, except for that in PF in June. These differences in bacterial metabolic diversity agree with the plant diversity patterns (Fig. 4a,b). Indeed, there is a positive linear correlation ($y = 0.085x + 2.640$, $r^2=0.834$, $t=7.090$, $p<0.001$) between plant species (x) and bacterial metabolic (y) diversities. Bacterial and fungal diversities in T soil sampled in December were the highest with significant differences relative to those in the other succession stages of the same season (Fig. 4c), but the soil bacterial metabolic diversity in T was the lowest among all four succession stages in the two

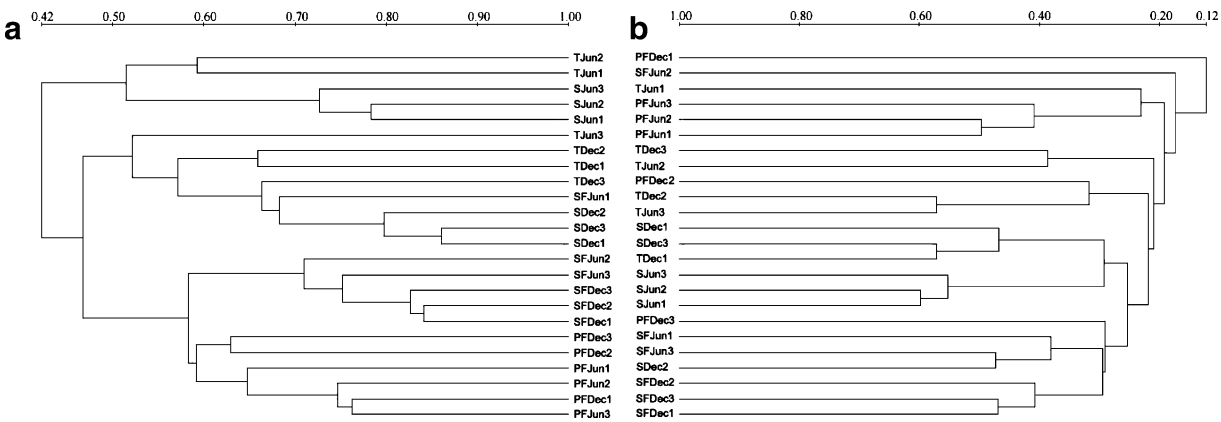

Fig. 2 UPGMA cladograms based on Dice similarity of bacterial (**a**) and fungal (**b**) community composition of soils from different vegetation successional stages and seasonal sampling times (for abbreviations and PCR-DGGE profiles see Fig. 1)

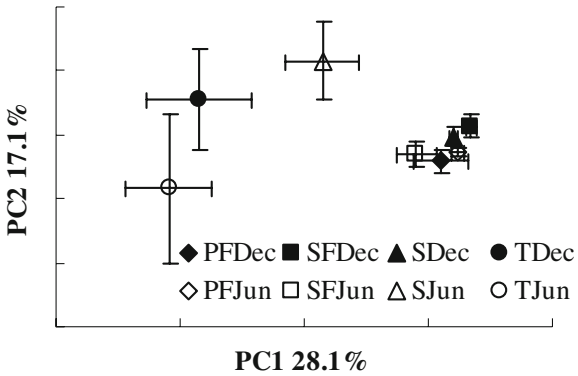

Fig. 3 Ordination biplot of a principal component analysis on the substrate utilization patterns of microbial communities from four vegetation successional communities derived from a Biolog_Eco plate assay. Shown are the means of three replicates with associated standard errors. For abbreviations of succession stages see Fig. 1

sampling seasons (Fig. 4b). In summer the bacterial diversities in all successional stages showed no significant differences among succession stages, but the soil fungal diversity in SF was significantly higher than in all other succession stages (Fig. 4c).

The soil bacterial and fungal diversity in T, and bacterial diversity in S, differed significantly between seasons (t-tests: $p<0.01$, $p<0.01$, $p<0.05$, respectively). However, soil microbial taxonomic diversities in SF and PF, and fungal diversity in S had no significant seasonal shifts (Fig. 4c).

The Pearson's correlation analysis indicated that there was a strong positive correlation between bacterial and fungal Shannon diversities (Fig. 5). No significant correlations were found between soil microbial taxonomic and metabolic diversity (data not shown).

Discussion

Plant diversity and bacterial metabolic diversity

Aboveground and belowground components of terrestrial ecosystems have been shown to be closely linked (van der Heijden et al. 2008; Wardle et al. 2004). In our study, higher plant diversity correlated positively with higher bacterial metabolic diversity (Fig. 4a & b). A possible mechanism behind this finding could be that higher plant diversity generally leads to higher plant biomass productivity (Cardinale et al. 2007), which could then lead to larger amounts of C entering the soil system (Liu et al. 2007). In fact, we found exactly the same ranking for plant diversity and SOM among the four succession stages (Table 2). Alternatively, an increase in plant richness and diversity could have led to greater resource heterogeneity in the soil (Rodríguez-Loinaz et al. 2008). Although the relationship between plant species diversity and plant productivity is still controversial (Chris and Richard 2006; Pärtel et al 2007), there are many published studies reporting higher productivity of species-rich relative to species-poor plant communities (Tilman and Downing 1994; Tilman et al. 1997).

Taxonomic diversity of plants and soil microbes

Although vegetation has significant effects on soil microbial taxonomic diversity (Table 3), the diversities of bacteria and fungi do not positively correlate with plant diversity along the vegetation succession (Fig. 4). Bacterial and fungal diversities were highest in T in December, but the plant species diversity was lowest in T among all four succession stages (Fig. 4a).

Table 3 Two-way ANOVA tables for the effect of vegetation type, season of sampling, and their interaction on bacterial and fungal taxonomic, and bacterial metabolic diversities (H)

Main effect	H′bac			H′fungi			H′meta		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Vegetation	3	3.746	*	3	15.125	***	3	90.061	***
Season	1	29.001	***	1	19.505	***	1	0.176	NS
Vegetation×Season	3	4.922	*	3	8.566	**	3	5.358	*
Residual	16			16			16		
Total	23			23			23		

* Significant levels: ***$P<0.001$; **$P<0.01$; *$P<0.05$; NS not significant ($P>0.05$)

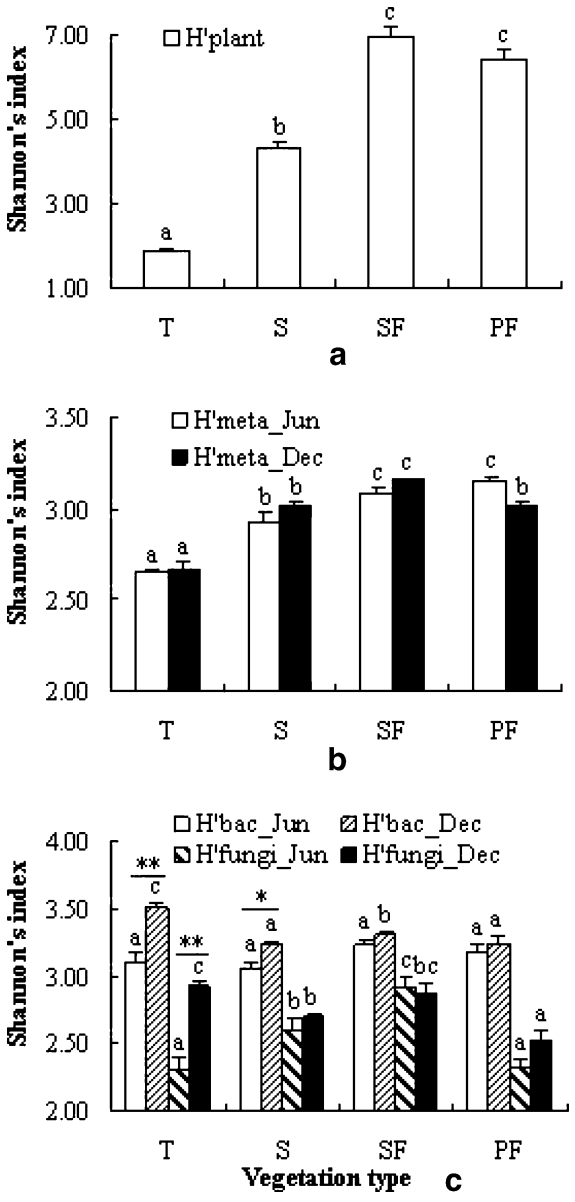

Fig. 4 Plant species (**a**) and soil bacterial metabolic Shannon diversities (**b**) and soil bacterial and fungal taxonomic diversities (**c**) at two sampling times in a secondary vegetation succession. Values represent the means of three replicates with associated standard errors. Different letters indicate a significant difference at $p<0.05$, according to Duncan tests. Significant seasonal variation of soil bacterial and fungal taxonomic diversities (**c**) for four soil microbial communities by the independent samples t-test are shown as a short line and * (**$P<0.01$; *$P<0.05$), all non-significant seasonal diversity comparisons were omitted. For vegetation type abbreviations see Fig. 1

As the main primary producers in terrestrial ecosystems, plants are thought to have profound effect on soil communities and processes, especially those in the rhizosphere (Bever 1994, 2002; Wilson and Hartnett 1997). Consequently, changes in nutrient quality and availability caused by changes in plant diversity are expected to alter the number, activities and diversity of soil microorganisms (Hooper et al. 2000). Although each stage of succession was characterized by a particular plant species assemblage, Chabrerie et al. (2003) found that microbial communities show a degree of resilience with respect to changes in plant community composition. Similarly, our findings show that there is no direct link between plant and microbial taxonomic diversity.

Taxonomic structure of bacterial and fungal communities

The soil bacterial communities in T and S were more similar to each other than those in SF and PF. This indicates that soil bacterial communities may show successional changes that follow those in aboveground vegetation as reported by Kardol et al. (2007). More variability in the soil bacterial communities of replicate samples from T and S than samples from SF and PF may indicate that there were more different niches in the oligotrophic environments of T and S (Table 1). In contrast to bacteria, the fungal communities generally showed more variability and more distinctly dominant ribotypes, which is in agreement with the results of Costa et al. (2006).

The significant positive correlation between the bacterial and fungal diversities points at the existence

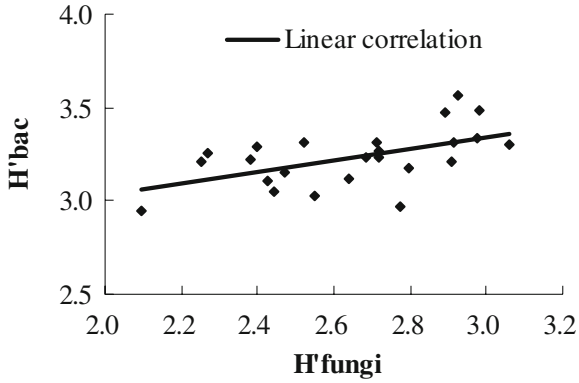

Fig. 5 Relationship fungal and bacterial diversities (H'). Linear correlation: $y = 0.319x + 2.387$ (r^2=0.305, p=0.005)

of a close relationship between these two groups of microorganisms in the studied soils. Fungi can be important in shaping the soil bacterial assemblages in trophic web interactions (Singh et al. 2008). Artursson et al. (2005) demonstrated selection of distinct bacterial populations following inoculation of the AM fungus *Glomus mosseae* in soils and, a direct effect of mycelia exudates on bacterial assemblage was found in culture (Toljander et al. 2007). Moreover, fungi can act as vectors for bacterial migration (Kohlmeier et al. 2005).

Relationship between bacterial taxonomic diversity and metabolic diversity

No positive correlations between taxonomic and metabolic diversity were found, which is in accordance with some previous studies. For example, O'Donnell et al. (2001) suggested that factors other than community structure, such as soil pH, were more important in regulating metabolic activity. The most likely explanation for a lacking correlation between bacterial taxonomic and metabolic diversity is that bacteria and fungi possess dormant growth stages (Chabrerie et al. 2003) and that DNA approaches capture the whole and not only the physiologically active communities. In general, depending on the conditions of nutrient supply, only a fraction of the microbes in soils are active (Chabrerie et al. 2003), whereas the large majority is either dormant or dead (McGill et al. 1986). This inactive part of the total microbial communities contributes to the soil 'memory' (Chabrerie et al. 2003). In agreement with this, Sharma et al. (2006) found that napA and nirS DGGE profiles derived from DNA showed no changes, whereas those from cDNA showed a clear succession of denitrifying bacteria. Moreover, different groups of soil microbes show metabolic redundancy, which means that any loss of microbial taxonomic diversity does not necessarily have to result in loss of metabolic functions (Wertz et al. 2006).

Seasonal variation

Bacterial and fungal taxonomic diversities differed (Fig. 4c; Table 3) between seasons, which agrees with previous findings of seasonal shifts in microbial communities (Schmidt and Lipson 2004; Monson et al. 2006; Wallenstein et al. 2007). Seasonal variation in the supply of substrates for microbial growth was found to shift from carbon polymers/phenolics (winter) to proteins (snowmelt) to rhizodeposition (summer) (Schmidt et al. 2007). However, bacterial metabolic diversity in this study did not differ between winter and summer (Table 3). The observation of seasonal shifts in microbial taxonomic diversity, but not in metabolic diversity may point at the existence of changes in microbial dormancy and metabolic redundancy. Occurrence of seasonal variation of microbial taxonomic differed between succession stages as assessed by independent t-tests (Fig. 4c). We may hypothesize that in the more oligotrophic T soil (Table 1) more r-selected microbes with more pronounced seasonal population shifts were favored, leading to the significant seasonal differences in taxonomic diversity. On the contrary, we may imagine that the more steady and copiotrophic soil environments in SF and PF favored more K-selected organisms (Fierer et al. 2007), showing more constant community compositions over vegetation seasons. However we found bacterial metabolic diversity in PF to differ significantly between seasons. Seasonal litters fall in the deciduous forest PF may explain seasonal differences in bacterial metabolic diversity in this succession stage, but not in the evergreen forest SF.

As it appears from unchanged bacterial metabolic diversities in T, S and SF, litter leaching (Qiu et al. 2005) and organic matter decomposition (Schmidt et al. 2007; Zogg et al. 1997) during the plant growth season (summer) were no determinant factors of bacterial activities in our study sites. Similarly, changes in soil moisture content that may have occurred were previously found not to explain the activity of microbial communities (Bossio et al. 1998; Krave et al. 2002). Irrespective of the season, differences in plant community composition appeared to explain differences in bacterial metabolic diversity in the present and a previous field study (Rogers and Tate 2001).

The microbial community from the S succession stage is special in so far as the bacterial taxonomic diversity, indeed, showed significant seasonal changes (Fig. 4c), whereas neither the fungal taxonomic, nor the bacterial metabolic diversity did so. Compared to T the soil environment of S appears to have been relatively more seasonally stable, because in T there were significant seasonal changes in bacterial and fungal taxonomic diversities. No seasonal differences

were found for the SF and PF succession stages. This shows that with plant succession development metabolic functioning of soil microbial communities become more stable over plant growth seasons and that bacteria are more susceptive than fungi to seasonal fluctuations in the soil environment. With respect to bacterial CLPPs it is interesting to note that the succession stage S lay in between of the old (SF and PF) and young (T) succession stages in summer and was more similar to SF and PF in winter (Fig. 3). This may show that competition among metabolic functional groups could be important in successionally intermediate bacterial communities when the availability of nutrient resources changes over the seasons.

Summary

In summary, this study showed that bacterial metabolic, but not taxonomic diversity can follow an increase in plant diversity. Moreover, we found evidence pointing towards an increased seasonal stability of soil bacterial metabolic diversity over successional plant community development. Seasonal differences in microbial taxonomic and metabolic diversity were more often found in younger than older plant succession stages. Finally, our field survey showed that bacterial and fungal taxonomic diversity can be correlated. We conclude that apparent anthropogenic impacts on the integrity of plant community diversity may be paralleled by changes to the soil microbial community and that metabolic functioning of bacterial communities in disturbed environments (reflected by younger succession stages) may become less stable over the plant growth season, with yet unknown consequences.

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