Short Communication

Effects of Plant Litter Type and Additions of Nitrogen and Phosphorus on Bacterial Community-Level Physiological Profiles in a Brown Forest Soil

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Effects of plant litter type (larch needle-leaves, mixed broad-leaves, and sasa green leaves) and nutrient addition (nitrogen and phosphorus) on bacterial community-level physiological profiles (CLPPs) of a forest soil were examined using BIOLOG EcoPlates™. Both the litter and nutrient additions significantly increased color development in most of the wells in the BIOLOG microplates, with the effect of the latter being especially great for soils amended with plant leaves low in nutrients. Nitrogen addition to soils decreased the color development of some nitrogenous substrates. Litter type had a dominant effect on the CLPPs. The addition of nitrogen also strongly affected the CLPPs.

Key words: bacterial community-level physiological profile, BIOLOG, plant litter, nitrogen, phosphorus

Decomposition of litter and soil organic matter, primarily mediated by soil microorganisms, enables the recycling of nutrients in ecosystems. Therefore, many studies have addressed the microbial decomposition process in soils (9). In forest ecosystems, nitrogen and phosphorus are the most common limiting nutrients (2). These two elements also have a substantial influence on the decomposition of plant litter, especially in its early stages (1), though their influence varies depending on the chemical composition of the litter. These influences would be related to the composition and function of the microbial community. However, little information is available concerning the influences of litter type and levels of nitrogen and phosphorus on the functional diversity of soil microbial communities. A detailed elucidation of the process would deepen our understanding of nutrient cycling in forest ecosystems. In the present study, we compared the effects of three types of leaf amendments on the community-level physiological profiles (CLPPs) of soil bacteria, the abundance of which reaches a peak faster than that of fungi after the addition of plant litter (9) because of their rapid growth. Also, we examined the changes in the CLPP in response to the addition of nitrogen and phosphorus, which are potential limiting nutrients in the early stage of litter decomposition, in a brown forest soil.

A soil sample was taken from the A horizon of brown forest soil (Cambisol) at an elevation of 780 m at Hora (36.23°N, 137.98°E), Matsumoto, Japan. The sample was sieved through a 2-mm mesh and well homogenized. A portion of the soil was air-dried for chemical analyses, while the remainder was maintained field-moist at 4°C. The soil characteristics were as follows: pH, 4.9; organic C, 5.0%; total N, 0.30%; texture, light clay (36% sand, 32% silt and 32% clay).

Larch (*Larix kaempferi*) needle-leaves and mixed broad-leaves (composed of oak *Quercus serrata* and chestnut

Castanea crenata leaves) were manually collected from the L horizon. Green leaves of sasa (*Sasamorpha borealis*) were collected by stripping. The air-dried leaves were ground with a vibrating sample mill TI-100 (Heiko Seisakusho, Tokyo, Japan) and then used for a laboratory incubation experiment and leaf chemical analyses. Total C and N contents were measured with an NC analyzer. Total P content of leaf samples was measured by the vanadomolybdate method (6) after digestion with nitric acid. Organic materials in the ground leaf samples were fractionated into lipids, water-soluble polysaccharides, hemicellulose, cellulose, and lignin at Createrra (Tokyo) using the proximate analytical method of Waksman and Stevens (10) with some modifications (6). The characteristics of the leaf samples are shown in Table 1.

To evaluate the effects of adding the leaf samples, nitrogen, and phosphorus on the CLPP of the forest soil bacterial community, a laboratory incubation experiment was conducted. The forest soil sample was preincubated at 60% of the water holding capacity for two weeks at 22°C, and then each leaf sample (larch needle-leaves, mixed broad-leaves, and sasa leaves) was treated as follows: (i) soil+leaf sample, (ii) soil+leaf sample+N, and (iii) soil+leaf sample+P (*n*=1 for each treatment). After the addition of the ground leaf (60 mg g⁻¹ soil), nitrogen (0.35 mg N g⁻¹ soil as a solution of NH_4Cl, adjusted to pH 4.9) and phosphorus (0.07 mg P g⁻¹ soil as a solution of Na_2HPO_4, adjusted to pH 4.9) to the preincubated soil, these samples were well mixed. As a control, un-treated

Table 1. Properties of leaf samples (mg g⁻¹ on a dry weight basis)

	Larch	Sasa	Mixed broad leaves
Total C	542	466	460
Total N	10.6	23.1	9.1
Total P	0.67	2.6	2.1
Lipids	87	105	77
Water-soluble polysaccharides	76	87	136
Hemicellulose	116	114	133
Cellulose	109	544	213
Lignin	405	115	307

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soil was also incubated. The treated samples (20 g of soil on a dry weight basis) were incubated in loosely capped bottles of 100 mL at 22°C, and distilled water was occasionally added to maintain the soil moisture at a constant level. After a two-week incubation, the soil bacterial CLPP (physiological profile of bacterial community derived from the original soil and the plant leaves employed) was examined.

The CLPP of the soil sample was examined using BIOLOG EcoPlates™ (Biolog, Hayward, CA) containing 31 different environmentally relevant C sources and a water well. In this analysis, the degree of utilization of each C source is measured based on the color formation from a redox indicator (tetrazolium dye), which is caused by formazan production in the presence of respiring bacteria (3). The soil suspensions in water (1:10 w/v) were dispersed with a Warring blender (10,000 rpm, 3 min), and then serially diluted to 10^{-3}. The BIOLOG plates were then inoculated with 150 μL of these suspensions. The plates were incubated for 5 days at 28°C. Color formation was measured every 24 h with a microplate reader (MicroLog™ System Release 4.20.04, Biolog) at 590 nm and 750 nm. The values used in each well were the 590 nm values (color development plus turbidity) minus the 750 nm values (turbidity), after correcting for the reading in the water well at these wavelengths. The data at 48 h were used in the analysis, because the treatment effects were most apparent in this data set. The average absorbance among three replicates of the 31 substrates in each plate was calculated. The overall color development in each plate was expressed as the average well color development (AWCD). Also, the substrates were divided into 6 functional guilds of compounds [amines (n=2), amino acids (n=6), carbohydrates (n=10), carboxylic acids (n=7), polymers (n=4), and phenolic compounds (n=2)] (5), and the average absorbance for all wells within each guild was calculated.

Two-way analysis of variance (two-way ANOVA) was carried out to determine if there was a significant effect of nutrient addition and leaf type on the AWCD. Principal component analysis (PCA) was used to describe the overall patterns of substrate use. All variables were standardized by performing a PCA on the correlation matrix of the variables. We excluded 5 substrates in the PCA because they showed activity in 1 or none of the 10 samples. The first two principal components were analyzed by two-way ANOVA to examine the effects of nutrient addition and leaf type. Since the CLPP was compared among the different treatments (i.e., leaf type and nutrients), normalization by AWCD was not performed (3). However, similar results were obtained when the value of each individual well was divided by the AWCD of the entire plate and then used in the PCA (not shown). These analyses were done using SRISTAT (Social Survey Research Information, Tokyo).

The addition of plant leaf samples, nitrogen, and phosphorus had remarkable effects on the AWCD in the brown forest soil. The activity for all the substrates was significantly affected by leaf type (two-way ANOVA, $p<0.01$; Fig. 1(a)). Among the leaf samples, green sasa leaves with high levels of nitrogen and cellulose (Table 1) caused the greatest increase in the AWCD. Although the larch needle-leaves and mixed broad-leaves used were not fresh litter, they also
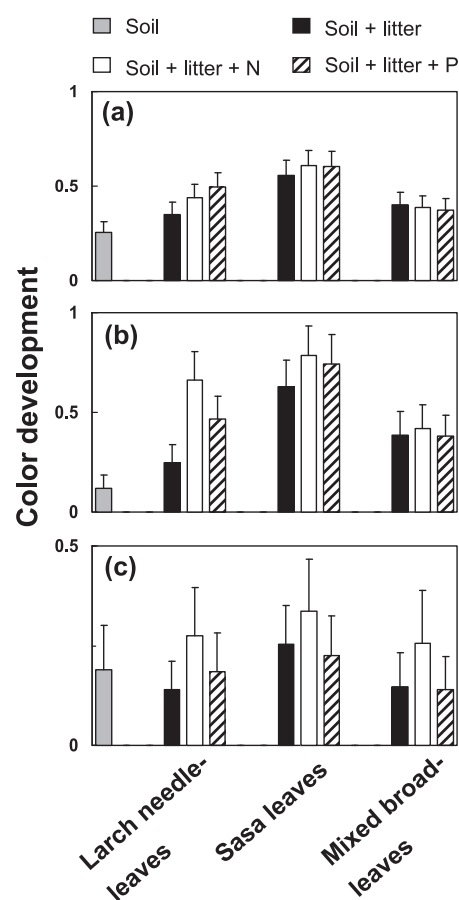

Fig. 1. Average well absorbances in the BIOLOG microplates: (a) all the substrates (n=31), (b) carbohydrates (n=10), and (c) polymers (n=4). Values are means±standard errors. Leaf type had a significant influence on activity for all the substrates (two-way ANOVA, $p<0.01$) and for carbohydrates ($p<0.05$), whereas polymers were influenced by both leaf type (p=0.01) and nutrient addition ($p<0.01$).

increased the AWCD (Fig. 1(a)). In these samples, microorganisms probably utilized easily degradable components uncovered by the grinding with a mill. When the substrates were divided into 6 guilds, the increase in the AWCD with the addition of leaf samples (without N or P) was greatest for the carbohydrates (3.5-fold), followed by the amines (2.2-fold). In two-way ANOVA, the activity for carbohydrates was significantly affected by leaf type ($p<0.05$; Fig. 1(b)), and the polymers were influenced by both leaf type (p=0.01) and nutrient addition ($p<0.01$) (Fig. 1(c)). In the other guilds, only a weak influence of leaf type was observed for the phenolic compounds (p=0.08), and nutrient addition for the amino acids (p=0.09). That the increase in the AWCD was greatest for the carbohydrates is likely due to the fact that the utilization of carbohydrates is important during the early stages of litter decomposition in soils (7). Garland (3) also suggested that carbohydrate utilization appears to respond most significantly to perturbations in bacterial communities. It is worth noting that the addition of nitrogen decreased the color development for some amines and amino acids (the decrease was 30.5% for putrescine, 38.8% for L-arginine, 33.8% for L-asparagine, and 71.7% for L-serine) in the soils amended with larch needle-leaves and mixed broad-leaves. Two-way ANOVA also detected a slight effect of nutrients
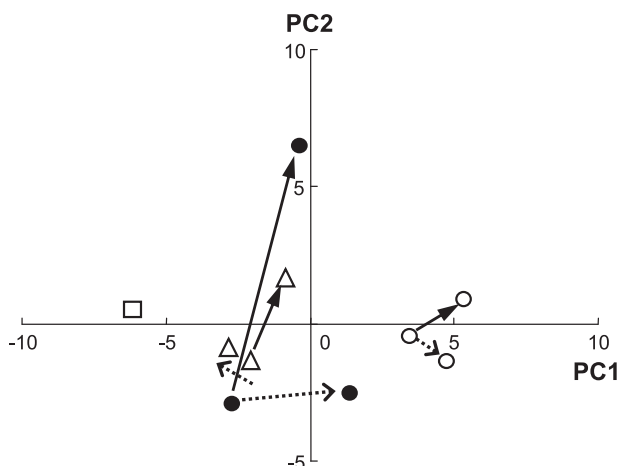
Fig. 2. Principal-component diagram of absorbance data from the BIOLOG microplates: ●, soil amended with larch needle-leaves; △, soil amended with mixed broad-leaves; ○, soil amended with sasa leaves; and □, control soil. The solid arrow indicates the effect of nitrogen, and the dashed arrow shows effect of phosphorus.

Table 2. Correlation of carbon source variables with principal components in the soil samples

Carbon source	r
PC1	
Carbohydrates	
D,L-α-Glycerol phosphate	0.890
D-Cellobiose	0.852
N-Acetyl-D-glucosamine	0.829
Glucose-1-phosphate	0.793
D-Galactonic acid lactone	0.766
β-Methyl-D-glucoside	0.757
D-Mannitol	0.734
D-Xylose	0.719
Carboxyl acids	
D-Glucosaminic acid	0.963
Itaconic acid	0.817
Pyruvic acid methyl ester	0.764
Amino acids	
L-Phenylalanine	0.947
L-Threonine	0.759
Phenolic compounds	
4-Hydroxy benzoic acid	0.882
Polymers	
Glycogen	0.778
PC2	
Amino acids	
L-Asparagine	−0.876
L-Arginine	−0.859
L-Serine	−0.823
Carbohydrates	
i-Erythritol	0.840
Polymers	
Tween 40	0.853

on the AWCD for the amino acids (p=0.09), and a remarkable effect of nitrogen was observed in the PCA as described below. Abundant nitrogen due to the addition of nitrogen may be responsible for the decline in the metabolism of these nitrogenous substrates in the bacterial community.

The results of the PCA are shown in Fig. 2 and Table 2.

The first principal component (PC1) accounted for 47% of the variation with PC2 accounting for 25%. Throughout the entire data set, litter type had a dominant effect on the change in the CLPP of the brown forest soil (Fig. 2). The addition of leaf litter increased the PC1 value, and soils amended with the different leaf types were separated along the PC1 axis. A significant influence of leaf type on PC1 was also observed in two-way ANOVA (p<0.01). All the substrates were positively correlated with PC1 (only strong correlations are shown in Table 2). These results suggested PC1 to be associated with the nutrient status of the soils. The points representing soils amended with the sasa leaves lie to the right of the origin and are situated furthest from the control soil point. This result suggests the largest influence of the sasa leaves amendment on the CLPP in the soil, which is consistent with the AWCD result (Fig. 1(a)). Nitrogen increased the PC2 value for all three leaf types (Fig. 2). A weak influence of nutrient addition on PC2 was found in two-way ANOVA (p=0.12). All the substrates that were strongly negatively correlated to PC2 were amino acids (Table 2). It is known that the addition of nitrogen accelerates plant litter decomposition in the early stages (11). According to Güsewell and Verhoeven (4), decomposition is enhanced by added nitrogen when the litter contains less than 11 mg g^{-1} nitrogen and more than 0.3 mg g^{-1} phosphorus. Hence, for the larch leaves and mixed broad-leaves used in the present study, the addition of nitrogen might have accelerated the decomposition process, and this might reflect the large shift in the CLPP for the two leaf samples (Fig. 2) and the largest increase in the AWCD for carbohydrates and polymers (Fig. 1). For the larch leaves, the CLPP was also strongly impacted by phosphorus (Fig. 2) and the highest AWCD for all the substrates was observed for the soil amended with phosphorus (Fig. 1(a)). This might be due to the deficiency in phosphorus for decomposing microorganisms, because the N/P ratio on a mass basis of the larch leaves (N/P=16) was much greater than the criterion of N/P=9 (8), above which the addition of phosphorus would accelerate the decomposition of the litter.

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