

## Nitrogen transformations in the rhizosphere of different tree types in a seasonally flooded soil

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### ABSTRACT

Plant roots strongly influence C and N availability in the rhizosphere via rhizodeposition and uptake of nutrients. An *in situ* rhizobox approach was used to compare rhizosphere effects of different tree species and clones on N cycling under seasonally flooded soil. We examined N mineralization and nitrification rates, inorganic N, and microbial biomass C (MBC) and N (MBN) in rhizosphere and bulk soils of three poplar clones, alder, and willow plantations in southeast China. Significant differences in soil pH, total N, soil organic C, MBC, MBN, and MBC/MBN were found between bulk and rhizosphere soils except alder. Compared to bulk soil, the net N mineralization and nitrification rates in rhizosphere soil across all tree species and clones increased by 124–228% and 108–216%, respectively. However, NO<sub>3</sub><sup>-</sup>-N was depleted in the rhizosphere soil mainly owing to the root uptake and rhizosphere microbial immobilization. The magnitude of rhizosphere effects on N transformations was considerably different among the tree species studied. Of the tested ones, alder had the greatest rhizosphere effect on N transformation, indicating different capacities of tree species to facilitate N turnover in the rhizosphere.

**Keywords:** nitrogen mineralization; rhizosphere effect; tree species; nitrogen cycling; rhizobox

Rhizosphere processes affect the cycling and availability of nutrients. Some of the rhizosphere processes were studied extensively for agricultural crops and grasses grown under controlled conditions (Kuz'yakov et al. 2000, Jones et al. 2004), while rhizosphere processes under tree species in natural conditions are poorly understood. Limited research suggests that inorganic N in rhizosphere soils may be higher (De Neergaard and Magid 2001), lower (Wang et al. 2001, Warembourg et al. 2003), or similar (Yanai et al. 2003) to the bulk soil. Nutrient cycling in the rhizosphere of trees under field conditions may greatly differ from that of annual plants and tree seedlings due to their differences in nutrient requirement, soil conditions, as well as growth period (Gobran et al. 1998). However, most studies on rhizosphere nutrient cycling of

trees were conducted on seedlings in microcosms with few conducted in the field or forests (Phillips and Fahey 2006).

Since tree species are very different from root morphology and physiology as well as nutrient requirement (Wang et al. 2001, Jones et al. 2004), rhizosphere effects on nutrient cycling would vary with tree species (Garcia et al. 2005). Knowledge of differences in rhizosphere nutrient cycling among different species is fundamental for characterizing nutrient acquisition capacity of different tree species and for interpreting the influence of tree species on soil processes. However, only a few attempts were made to compare the rhizosphere nutrient cycling of different tree species (Wang et al. 2001, Kuz'yakov 2002, Phillips and Fahey 2006). Microbial biomass plays a significant role

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in nutrient transformation and ecosystem conservation under tropical and temperate climates, as turnover of microbial biomass influences the amount of N being stored or released, and thus availability of N for plant uptake (Wardle 1998). Moreover, rates of net mineralization and nitrification during laboratory or field *in situ* incubation provide an effective indicator of N availability (Hart et al. 1997). Therefore, there is a strong need for more field studies on rhizosphere nutrient cycling under different climates and tree species for providing more realistic views of rhizosphere processes (Gobran et al. 1998, Fang et al. 2013).

In the river channel of the mainstream of the mid-lower Yangtze River, there is a large number of floodplains which are valuable land resources (Liu et al. 2012). Afforestation is recommended as a viable option for ecological restoration and enhancement of biological diversity in such less favorable soils that are often periodically flooded. The primary objective of this study was to quantify and compare the effects of different tree species on soil N transformations in the rhizosphere under seasonally flooded field conditions. We hypothesized that (1) rhizosphere effects would lead to increased N mineralization due to enhanced microbial activity in the close vicinity of roots for all three species and (2) the magnitude of these effects would differ among the species or gene types due to differences in intrinsic biological characteristics.

## MATERIAL AND METHODS

**Site description and plantation establishment.** This study was conducted near Ma-an-shan, Anhui in southeast China. The study site (31°41'N, 118°26'E) is located at the eastern lower reaches of the Yangtze River with annual seasonal flooding for about one month between June and July. This floodplain of the Yangtze River was originally covered by common reed (*Phragmites australis*). In December 2007, the site was mounded by an excavator to form 'ridge and trough' for planting trees. More detailed climate and soil conditions of the site were described in Liu et al. (2012).

Three poplar clones (Nanlin-895, NL-80351 and 75), one type of alder (*Alnus trabeculosa*) and one type of willow (*Salix × jiangsuensis* cv. J799) were planted on the ridge in March 2008 using 1-year-old rooted seedlings with 3 × 5 m planting spacing (Liu et al. 2012). Three planting patterns were established,

including poplar-willow mixture (1:1 ratio), poplar-alder mixture (1:1), and poplar-willow-alder mixture (1:0.5:0.5). A randomized block design was used with three replications for the three planting patterns. After one year following the planting, the average survival of the plantations was more than 97%.

**Rhizobox design, installation, retrieval and soil sampling.** Rhizoboxes in this study were made of two attached PVC cylindrical compartments that were separated by a 100 µm thick polyamide mesh with a 30 µm pore diameter (PA6366, 150-30WPW, Tiantai, Zhe-jiang, China). The upper compartment volume was 265 mL, while the lower compartment was 408 mL (Liu et al. 2012). The two compartments were packed with alluvia soil at a bulk density of 1.19 g/cm<sup>3</sup>. The soil was collected from about 10–15 cm depth near the respective trees to which the rhizoboxes were later installed (Liu et al. 2012). After packing, the top opening of the upper compartment was covered with a 1 mm plastic mesh which allowed tree roots to grow. The bottom of the lower compartment was covered with an a30 µm polyamide filter to prevent other plant roots from penetrating the rhizobox (Liu et al. 2012).

Twenty trees for each poplar clone, alder and willow were selected for installing the rhizoboxes between 40 cm and 60 cm from the tree trunk. All the rhizoboxes were buried at 20–30 cm depth with a 30 degree angle to mimic typical root growth habit (Liu et al. 2012). The rhizoboxes were installed in early April 2010, and retrieved in late October 2010.

After the rhizoboxes were retrieved from the field, the two rhizobox compartments were separated. After carefully removing the mesh, the soil packed in the lower compartment was pushed out slowly. Approximately the first 4 mm of soil in closest contact with the root mats were collected as rhizosphere soil; bulk soil samples were taken in 20–30 mm distance from the root mats.

Each sample was divided into two parts. One part was air dried for analysis of soil physico-chemical properties and for the incubation experiments. The other part was immediately stored at 4°C until the biological properties of the soil could be analyzed.

**Soil nitrogen mineralization.** Fresh soils were pre-incubated at 60% of water holding capacity (WHC) prior to the evaluation of N mineralization based on the differences in KCl extractable N with or without 30 days incubation. To test nitrogen mineralization, 5 g of pre-incubated soil were placed in a 25 mL glass beaker and six beakers with soil were prepared for each sample. Three of the

repetitions were extracted by 25 mL 2 mol/L KCl solution immediately. The other three of the repetitions were incubated in the dark for 30 days at 25°C in constant temperature ware (DYX-DHS-40 × 50, Zhejiang, China). During the incubation, in order to prevent water loss, soil moisture was checked every 2–3 days by weighing and restored at 60% WHC whenever needed, drop by drop. After incubation, soil in each beaker was transferred quantitatively to a 50 mL centrifuge tube with 25 mL 2 mol/L KCl solution. After shaking for 1 h at 25°C, the suspension was filtered (quantitative filter paper, No. 203), followed by quantification of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^+\text{-N}$  using a continuous-flow auto-analyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Hamburg, Germany).

Potential net N mineralization rate was estimated by inorganic N accumulated during incubation, which was calculated as

$$[(\text{NH}_4^+\text{-N} + \text{NO}_3^+\text{-N})_{\text{after incubation}} - (\text{NH}_4^+\text{-N} + \text{NO}_3^+\text{-N})_{\text{pre-incubation}}]$$

over the 30-day incubation. Potential net nitrification rate (%) was calculated as

$$\text{NO}_3^+\text{-N}/(\text{NH}_4^+\text{-N} + \text{NO}_3^+\text{-N})_{\text{after incubation}} \times 100.$$

**Laboratory analysis.** Soil pH was measured in a soil-water suspension (soil:water = 1:5 w:v) using an automatic acid-base titrator (SH/T0983, Zhejiang,

China). Soil total nitrogen (TN) was determined by digesting the samples following the Kjeldahl method (Bremner and Mulvaney 1982) with the N concentration in the digest measured using an auto analyzer III-AA3 (Bran + Luebbe GmbH, Munich, Germany). Soil organic carbon (SOC) was determined by  $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$  oxidation method (Nelson and Sommers 1982), and measured using a LiquiTOC II analyzer (Elementar, Frankfurt, Germany).

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) was measured by using the fumigation-extraction method (Vance et al. 1987).

**Statistical analyses.** Paired sample *T*-test was used to compare the differences in soil variables between rhizosphere and bulk soil samples for each tree species separately ( $P < 0.05$ ). The magnitude of the rhizosphere effect was calculated as the percentage difference between paired rhizosphere and bulk soil samples for each soil variable. One-way analysis of variance (ANOVA) was performed to test the effects of tree species on the magnitude of rhizosphere effects, potential net N mineralization rate, or potential net nitrification rate. Comparison among means was made using the Duncan's multiple range tests at  $P < 0.05$ . All statistical analyses were performed using the SPSS statistical software package version 15 (SPSS Inc. 2005, Chicago, USA).

Table 1. Soil pH, total N (TN), organic C (SOC), microbial biomass N (MBN), microbial biomass C (MBC), and C:N ratio (MBC/MBN) in bulk and rhizosphere soils of different tree species and poplar clones

Tree species or clones	Sampling position	pH value	TN	SOC	MBN	MBC	MBC/MBN
			(g/kg)		(mg/kg)		
Nanlin-895	rhizosphere	8.02 ± 0.03	2.11 ± 0.03	3.98 ± 0.43	27.83 ± 2.62	108.83 ± 3.66	3.92 ± 0.82
	bulk soil	8.10 ± 0.05	2.06 ± 0.07	2.28 ± 0.14	17.91 ± 0.94	44.16 ± 3.23	2.47 ± 0.73
	<i>T</i> -test	**	*	**	**	**	**
75	rhizosphere	8.00 ± 0.02	1.89 ± 0.05	3.43 ± 0.26	25.22 ± 3.82	97.84 ± 2.95	3.88 ± 0.65
	bulk soil	8.09 ± 0.02	1.94 ± 0.06	2.21 ± 0.18	17.87 ± 0.87	52.67 ± 3.36	2.95 ± 0.46
	<i>T</i> -test	**	**	**	**	**	**
NL-80351	rhizosphere	8.01 ± 0.01	1.62 ± 0.15	2.98 ± 0.22	24.88 ± 1.86	77.84 ± 5.68	3.13 ± 0.53
	bulk soil	8.10 ± 0.01	1.49 ± 0.06	2.03 ± 0.50	19.44 ± 1.66	39.27 ± 3.88	2.02 ± 0.29
	<i>T</i> -test	**	**	**	*	**	**
Willow	rhizosphere	7.96 ± 0.05	1.47 ± 0.06	2.88 ± 0.36	24.11 ± 2.74	82.83 ± 4.71	3.46 ± 1.01
	bulk soil	8.04 ± 0.04	1.48 ± 0.17	1.91 ± 0.23	20.14 ± 0.43	36.27 ± 3.6	1.92 ± 0.92
	<i>T</i> -test	*	ns	**	ns	**	**
Alder	rhizosphere	8.07 ± 0.02	1.28 ± 0.11	1.98 ± 0.55	16.82 ± 1.45	43.84 ± 4.14	2.61 ± 0.74
	bulk soil	8.07 ± 0.02	1.26 ± 0.09	1.87 ± 0.16	15.70 ± 0.76	39.83 ± 3.66	2.54 ± 0.85
	<i>T</i> -test	ns	ns	ns	ns	ns	ns

Data represent the means ± SD (standard deviation). ns – no significant difference (by paired samples *T*-test). Significant differences between bulk and 352 rhizosphere soils at \* $P < 0.05$  and \*\* $P < 0.01$

Table 2. The magnitude of rhizosphere effect of different tree species and clones on soil variables (%)

Tree species or clones	SOC	MBC	MBN	MBC/MBN	Net N mineralization	Net nitrification
Nanlin-895	75 <sup>a</sup>	146 <sup>a</sup>	55 <sup>a</sup>	59 <sup>b</sup>	124 <sup>c</sup>	126 <sup>b</sup>
75	55 <sup>b</sup>	85 <sup>b</sup>	41 <sup>b</sup>	32 <sup>c</sup>	212 <sup>a</sup>	108 <sup>b</sup>
NL-80351	47 <sup>c</sup>	98 <sup>b</sup>	28 <sup>bc</sup>	55 <sup>b</sup>	140 <sup>b</sup>	142 <sup>b</sup>
Willow	51 <sup>c</sup>	142 <sup>a</sup>	20 <sup>c</sup>	80 <sup>a</sup>	169 <sup>b</sup>	150 <sup>b</sup>
Alder	6 <sup>d</sup>	10 <sup>c</sup>	7 <sup>d</sup>	3 <sup>d</sup>	228 <sup>a</sup>	216 <sup>a</sup>

The magnitude of rhizosphere effect was calculated by the formula: [(rhizosphere parameter – bulk soil parameter)/bulk soil parameter] × 100. Values in columns with different superscript letters mean significant differences among tree species at  $P < 0.05$ . SOC – soil organic carbon; MBC – microbial biomass C; MBN – microbial biomass N

## RESULTS

The pH values were not significantly different between bulk and rhizosphere soils in alder plantations, but were significantly lower in rhizosphere soil of poplar clones and willow than the respective bulk soils (Table 1). Total N concentration and MBN were significantly higher in the rhizosphere soil than in the bulk soil for poplar clones, but rhizosphere effects on TN were not significant for willow and alder trees. Rhizosphere effects on SOC, MBC and MBN were significant for most of the tested tree species, with the greatest rhizosphere effect in the Nanlin-895 poplar clone and the smallest in alder (Table 2). The magnitude of the rhizosphere effects on microbial C:N ratios were also significantly different among tree species and clones, which was in the order of willow > Nanlin-895 > NL-80351 > 75 > alder (Table 2).

Concentrations of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were significantly different between rhizosphere and bulk soils for all tree species (Figure 1a,  $P < 0.05$ ). Compared

to the mean of bulk soil,  $\text{NO}_3^-$ -N concentration in the rhizosphere soils of Nanlin-895, 75, NL-80351, willow and alder decreased by 42.2, 31.7, 35.3, 46.8 and 62.2%, respectively. However,  $\text{NH}_4^+$ -N concentration significantly increased in rhizosphere soil as compared with bulk soil in all tree species and clones, and the magnitude of the rhizosphere effects on  $\text{NH}_4^+$ -N concentration were in the order of 75 > NL-80351 > Nanlin-895 > willow > alder.

Significantly higher N mineralization and nitrification rates in the rhizosphere than the bulk soils were observed for all tested tree species and clones (Table 2, Figure 1b). Rates of N mineralization and nitrification increased by 124–228% and 108–216%, respectively, across the tree species and clones. However, N mineralization rates were also significantly different among the rhizosphere soils across tree species and clones (Figure 1b,  $P < 0.05$ ). The net N mineralization rates in rhizosphere of 75, Nanlin-895, NL-80351 and alder were 79.1, 38.6, 47.3 and 31.2% greater than that of willow, respectively.

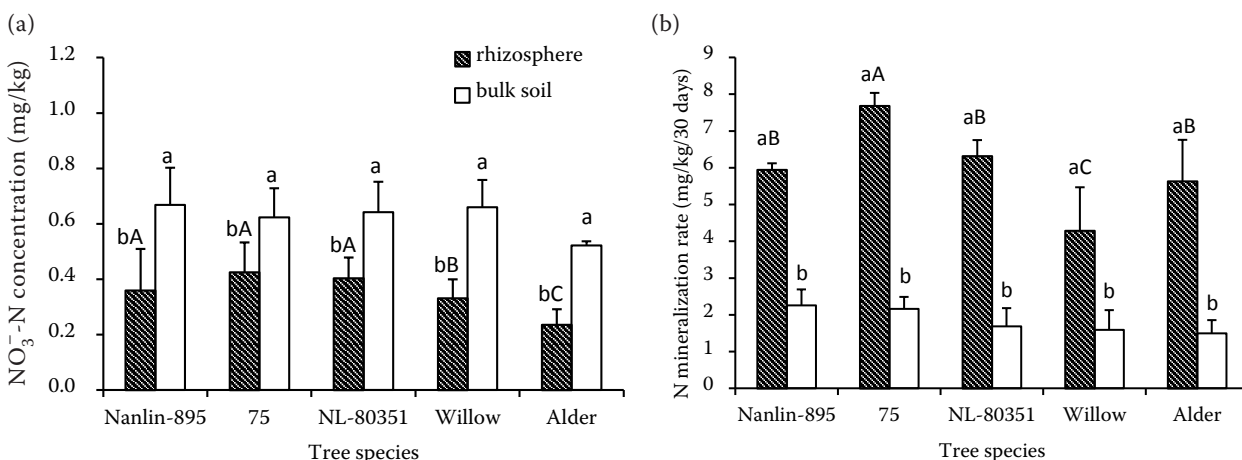


Figure 1. (a) Concentrations of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N, and (b) potential net N mineralization in bulk and rhizosphere soils of different tree species and clones (mean ± standard deviation). Significant differences between bulk and rhizosphere soils for the same tree species and clones are indicated by different lower case letters, while the differences among different tree species or clones in the rhizosphere soil are indicated by different capital letters ( $P < 0.05$ )

## DISCUSSION

**Rhizosphere effects on N mineralization and availability.** The obtained data supported our hypothesis that N mineralization in the rhizosphere would be promoted by rhizosphere effects of all tree species. The simultaneous increases in potential net N mineralization rate and MBN in rhizosphere soil suggest that tree roots stimulated gross N mineralization, as net N mineralization rate is the difference between gross N mineralization and N immobilization (which went to the MBN). In general, enhanced gross N mineralization in rhizosphere soil primarily resulted from the rhizosphere 'priming effects' on soil organic matter decomposition (Ehrenfeld et al. 1997, Phillips and Fahey 2006). Nitrogen transformation is tightly coupled with soil organic matter decomposition that can be strongly accelerated by rhizodeposition (i.e., root exudates) activated microbial growth (Jackson et al. 2008). In addition, release of  $\text{NH}_4^+$  by soil faunal grazing of rhizosphere microbes has been proposed as another important mechanism for increased rhizosphere N availability (Jackson et al. 2008).

The greater net nitrification rate in rhizosphere soil did not result in accumulation of  $\text{NO}_3^-$ -N (Figure 1a). On the contrary,  $\text{NO}_3^-$ -N concentration decreased in rhizosphere soil compared to bulk soil, indicating that the uptake of  $\text{NO}_3^-$ -N by plants and rhizosphere microorganisms might have exceeded the rate of nitrification. It is suggested that rhizosphere microbial growth is often N-limited rather than C-limited, especially in N-limited soils. Further, rhizosphere C flux may reduce N availability through microbial N immobilization (Phillips and Fahey 2006).

Rhizosphere effects of trees on mineral N concentrations vary among different studies. For example, rhizosphere soils showed significantly higher  $\text{NH}_4^+$  but similar  $\text{NO}_3^-$  concentrations in rhizosphere soil when compared with bulk soil in a mature Douglas-fir (*Pseudotsuga menziesii*) stand (Turpault et al. 2005). On the other hand, concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in rhizosphere soil of Norway spruce (*Picea abies*) and European beech (*Fagus sylvatica*) seedlings were significantly lower than the bulk soils (Wang et al. 2001). Ehrenfeld et al. (1997) found that  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were not influenced by live roots in the mineral soil. Inconsistency in these reported observations may reflect differences in soils and plant species employed in these studies.

### **Species differences in rhizosphere effects.**

Consistent with our hypothesis, the magnitude of rhizosphere effects on nitrogen transformation varied among tree species. Under seasonally flooded soil conditions, alder roots were more effective in facilitating N transformation, which is likely related to nitrogen fixation and root exudates of the species (Cheng et al. 1996, Phillips and Fahey 2006). In the present study, tree species with contrasting nutrient requirement and root characteristics had similar patterns of rhizosphere effects on N mineralization rates and inorganic N concentrations, but the magnitude of rhizosphere effects varied considerably among tree species. Similar patterns of rhizosphere effects on nutrient cycling for different tree species were also observed in other studies (Wang et al. 2001, Phillips and Fahey 2006). For example, Wang et al. (2001) observed that Norway spruce had similar patterns but more pronounced rhizosphere effects on soil chemistry than European beech. Phillips and Fahey (2006) observed that the magnitude of rhizosphere effects on C and N mineralization rates and phosphatase activity ranged from 10% to 35% across tree species with different arbuscular mycorrhizal and ectomycorrhizal fungi in their roots.

Though there is limited quantitative information on root exudates of mature trees under natural conditions due to technical difficulties, experiments on tree seedlings revealed that tree species differ greatly in the amount and type of root exudates (Grayston et al. 1996, Zhang and George 2009). Different magnitude of rhizosphere effects on MBC, MBN and microbial biomass C:N ratio in the present study also suggests that the amount and quality of root exudates differed among tree species. Of the tree species studied, significantly lower microbial biomass C:N ratio in rhizosphere soil of alder may indicate that the stimulation of N mineralization may result from the altered rhizosphere microbial community structure rather than from increased total microbial biomass. Conversely, significantly increased MBC and unchanged microbial biomass C:N ratio in the rhizosphere soil of willow and three poplar clones suggest that enhanced N mineralization in rhizosphere soil may result from the increased rhizosphere microbial biomass. These results reflect mechanisms of rhizosphere effects on nutrient cycling and may differ among tree species. Though differences in root exudates were recognized as an important source responsible for differences in rhizosphere effects among different tree species (Kuzyakov

2002, Phillips and Fahey 2006), the underline physiological processes remain unknown.

In conclusion, the magnitude of rhizosphere effects on N transformations differed considerably among the tested tree species. Of the species tested, alder had the greatest rhizosphere effect on N transformation. The obtained results suggest that tree species have varied capacities facilitating N turnover in the rhizosphere. Higher N mineralization, higher nitrification rates, and accumulation of inorganic N in the rhizosphere could mean that the plant species have the desired aptitude to manipulate rhizosphere environment and microbial community to gain competitive advantage toward nutrient acquisition in support of growth and biomass production.

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