Carbon allocation of Chinese pine seedlings along a nitrogen addition gradient

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ABSTRACT

The response of carbon (C) allocation in pine seedlings to a gradient of nitrogen (N) addition was evaluated to examine the effects of increasing N deposition on forest trees. Seedlings of *Pinus tabulaeformis* were subject to N addition treatments (0–22.4 g N m^{-2} yr^{-1}) for 3 years and the short-term allocation of a ^{13}C photosynthetic pulse into leaves, stems, different order roots and soil was traced. Photosynthetic rate, roots respiration, biomass and N concentration of foliage and roots with root branching orders were also measured. It was found that the physiological performance of seedlings exhibited a modal response, peaking at 11.2 g N m^{-2} yr^{-1}. The highest N treatment (22.4 g N m^{-2} yr^{-1}) had detrimental effects on seedling physiological function (photosynthesis, root respiration) and was strongly decoupled from N concentrations in plant tissues. In agreement with the functional equilibrium hypothesis, root:leaf biomass ratio declined with N addition up to 11.2 g N m^{-2} yr^{-1}. The hypothesis states that a stable ratio of resource acquisition by shoots and roots is maintained in the face of constraints, so that one organ does not greatly outgrow the other and overall plant performance is optimized. This response was most pronounced when expressed on the basis of the lowest order roots. Allocation of C to fine roots compared to coarse roots increased with N availability up to the highest N treatment. Soil C flux declined significantly with excess N supply. The results support a "shared control" concept of root C allocation, in which the allocation of C to roots depends on both supply from the shoot and demand from the roots. This provides new insights into the effects of increasing soil N supply on pine seedling physiology and root carbon supply.

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1. Introduction

Plant growth in temperate ecosystems is commonly limited by the availability of nitrogen (N) (LeBauer and Treseder, 2008). Anthropogenic N deposition in these ecosystems is expected to affect carbon (C) assimilation capacity of plants by influencing photosynthetic rates and leaf area index (Taylor et al., 1993; Gastal and Lemaire, 2002; Yang et al., 2012). N deposition is also expected to effect the allocation of photosynthesis products between respiration and biomass production; ephemeral and long-lived tissues; and aboveground and belowground components (Litton et al., 2007). The changes of photosynthate allocation among plant organs could influence plant growth (Wendell et al., 1994) and net productivity (Gower et al., 1994), as well as biogeochemistry via influences on carbon sequestration and plant-atmosphere gas exchange. The stimulation of primary productivity by atmospheric CO_2 fertilization may be constrained by soil N availability (Norby et al., 2010). These observations emphasize the need for a mechanistic understanding of the response of forests to changing soil N availability.

Increased N availability caused by atmospheric N deposition or N addition could influence the amount and allocation of photosynthate to plant organs (Yang et al., 2012). Several studies have found that increased N availability reduces the root to shoot biomass ratio, which supported the functional equilibrium hypothesis of the C allocation to plant roots under changing N availability (Thornley, 1977; Wilson, 1988). This hypothesis states that a

stable ratio of resource acquisition by shoots and roots is maintained despite constraints to resource acquisition, so that one organ does not greatly outgrow the other and overall plant performance is optimized (Thornley, 1977). This concept underlies various models of plant growth (Hunt, 1990; Shipley and Meziane, 2002). Recent studies found that the biomass of fine root systems (first and second order roots) declined significantly with increasing N availability, but higher order root systems (third and above order roots) did not respond, suggesting a decrease in biomass ratios of fine roots to coarse roots with increasing N availability (Son and Hwan, 2003; Wang et al., 2013). The lower biomass ratio of fine roots to coarse roots does not necessarily indicate a lower proportion of carbohydrate allocation to fine roots because fine roots have a shorter lifespan and higher respiration rates than coarse roots (Pregitzer et al., 2002). Farrar and Jones (2000) investigated the physiological basis of the functional equilibrium hypothesis and controls on C allocation to roots, and noted that neither a "push" (from the shoot) nor a "pull" (from the root) fit all the experimental evidence. Results provided support for a concept of "shared control" in which the allocation of C to roots depends upon both supply from the shoot and demand from the roots. The need for additional experimental evidence, particularly for long-lived woody plants, is emphasized by the uncertainty surrounding ecosystem response in global change models (Thomas et al., 2013).

High levels of N addition have detrimental effects on vegetation in some terrestrial ecosystems. Aber et al. (1989, 1998) described a theory of N saturation in temperate forest ecosystems in which forest decline was predicted at high N loading. Over fertilization with N has been shown to effect pine seedlings (Nakaji et al., 2001), and long-term field experiments have demonstrated the sensitivity of conifers to high N loading (McNulty et al., 1996; Magill et al., 2000). Bauer et al. (2004) observed a decoupling between foliar N and maximum photosynthesis of *Pinus resinosa* at high N loading, as well as the accumulation of N in biochemical compounds, which is indicative of plant stress. Many industrialized regions in the world receive very high N loading potentially influencing forest health. However these mechanisms and the resulting implications for plant C allocation are not well understood.

The objective of the present study was to advance understanding of the responses of C allocation in pine trees to changes in soil N availability and to evaluate tree growth to N addition across the range from deficiency to excess N supply. We focused on the effects of N additions on the fine root system, as represented by root branching orders (Pregitzer et al., 1997). Pine seedlings were grown for four years in a field setting under five levels of N addition in the form of urea ($0–22.4 \, g \, N \, m^{-2} \, yr^{-1}$). This range was chosen to represent N deposition in polluted parts of China ($3–7.3 \, g \, N \, m^{-2} \, yr^{-1}$; Mo et al., 2008). A pulse-chase study of ^{13}C allocation to shoots, roots, and soil was conducted over 16 days to quantify the short-term patterns of response of C allocation to the N treatments. For the present study, we hypothesized that seedling growth would increase with N additions in the N-limited soils from the field site (Wang et al., 2013), but we anticipated a possible dysfunctional response due to overfertilization at high N addition levels. We also expected a decrease in seedling root:shoot ratio with increasing N availability. In support of the concept of shared control of C allocation to roots (Farrar and Jones, 2000), we anticipated that higher photosynthetic C supply would result in proportionally greater C export from foliage (push) as well as preferential supply of C to lower order roots that are most active metabolically and in nutrient acquisition (pull). Finally, we expected dysfunctional effects of excessive N addition to result in proportional reductions of C allocation to the shoot and to roots of different order.

2. Materials and methods

2.1. Research site

This research was carried out in an experimental field at the Institute of Soil and Water Conservation in Yangling, Shaanxi Province, China (108°4′ 27.95E, 34°16′ 56.24N). The site has a mainland monsoon type climate with average annual precipitation of 674 mm and a mean annual temperature of 13.2 °C. The soil is clay-loam, gray forest soil, which was collected from a planted Chinese pine (*Pinus tabulaeformis*) forest site. In the 0–20 cm soil layer clay, silt, and sand composition was 36.8, 24.3, and 38.9%, respectively. The organic matter content was $13.6 \pm 1.3 \, g \, kg^{-1}$ (mean ± SD, $n = 8$); total soil N content was $1.54 \pm 0.24 \, g \, kg^{-1}$; total soil P content was $1.42 \pm 0.38 \, g \, kg^{-1}$; and soil pH was 8.6 ± 0.3. Previous studies indicated that low soil N availability limited the growth of pine on this site (Wang et al., 2013).

2.2. Experimental design

The seeds of Chinese pine were sown and cultivated in a $5 \times 10 \, m$ seedling bed on the 28th of March 2007. In March 2008, 340 PVC pots with 35 cm diameter and 40 cm depth were filled with forest soil from the research site. Each pot was packed to a bulk density of approximately $1.14 \, g \, cm^{-3}$. The pots were put into 4 blocks and the urea (Fumin Agriculture Product Company, Xian, China) was dissolved into 10 ml distilled water and added to the pots evenly when it rained. This took place at the end of March or in early April of each year from 2008 to 2011. The maximum precipitation at this site did not cause any leaching of urea from the pot. The atmospheric N deposition rate at this site was found to be $2.06 \, g \, N \, m^{-2} \, yr^{-1}$, which is lower than most polluted regions in the south of China ($7.3 \, g \, N \, m^{-2} \, yr^{-1}$, Mo et al., 2008), but it is increasing rapidly. Fertilization treatment levels in the 4 blocks were as follows: 0 (as control), 2.8, 5.6, 11.2 and $22.4 \, g \, N \, m^{-2} \, yr^{-1}$ of urea (0, 0.57, 1.15, 2.3, 4.6 g urea pot^{-1} was dissolved into 10 ml distilled water, respectively). Sixty-eight pots were used for each N treatment (17 pots for each N treatment × 4 blocks). One, 1-year-old seedling was replanted at random in a pot and the pots were placed in the experimental field. In September 2011, soil total N contents of ^{13}C labeled samples and unlabeled sample for 0 (as control), 2.8, 5.6, 11.2 and $22.4 \, g \, N \, m^{-2} \, yr^{-1}$ treatments were 1.48 ± 0.18, 1.57 ± 0.23, 1.64 ± 0.31, 1.68 ± 0.25, $1.73 \pm 0.29 \, g \, kg^{-1}$ (mean ± SE, $n = 8$), respectively, and N contents of foliage and roots had significant response to N treatment (Table 1).

2.3. ^{13}C labeling

In September 2011, labeling was carried out from 9:00 am to 11:00 am in a greenhouse with $1000 \, \mu mol \, m^{-2} \, s^{-1}$ of photosynthetically active radiation (400–700 nm) at a temperature of 25 °C. The time of labeling was chosen to avoid the strong C sink associated with rapid shoot growth observed early in the growing season. Five pots from each N treatment were placed into a Plexiglas chamber ($100 \times 120 \times 100 \, cm^3$). Briefly, the chamber was sealed with tape and connected by tubing to a vial containing 2 g $Ba^{13}CO_3$ (99 atom%, Shanghai Engineering and Technological Research Center for Stable Isotope, Shanghai, China) and a flask containing 200 g $NaHCO_3$. Air within the chamber was stirred with a 30-cm-diameter fan. Total CO_2 concentration in the chamber was monitored with an LI-8100 CO_2 infrared gas analyzer (LI-COR, Inc.). When the initial CO_2 levels had declined to $100 \, \mu l \, l^{-1}$, HCl was added to the vial to increase the CO_2 concentration to $350–360 \, \mu l \, l^{-1}$. Prior to adding the HCl to the chamber, the light in the chamber was switched off for 4 min after