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A new isotope framework to decipher leaf-root nitrogen allocation and assimilation among plants in a tropical invaded ecosystem

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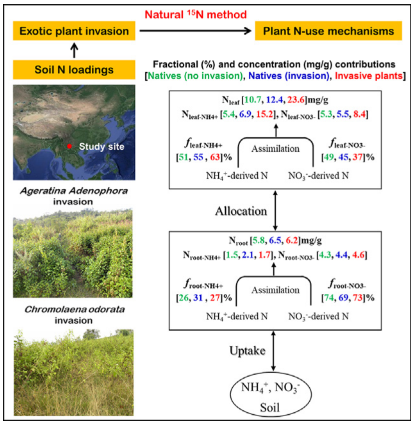
HIGHLIGHTS

- A new isotope method is established to decipher plant N-use strategies in a tropical site of SW China.
- Invasive plants allocated more NH_4^+-derived N to leaves than to roots than natives.
- Co-existing natives showed preferential N allocation to leaves over roots than natives under no invasion.
- Soil N especially NH_4^+ loading would promote exotic plant invasion.

GRAPHICAL ABSTRACT



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ABSTRACT

Exotic plant invasion is an urgent issue occurring in the biosphere, which can be stimulated by environmental nitrogen (N) loading. However, the allocation and assimilation of soil N sources between leaves and roots remain unclear for plants in invaded ecosystems, which hampers the understanding of mechanisms behind the expansion of invasive plants and the co-existence of native plants. This work established a new framework to use N concentrations and isotopes of soils, roots, and leaves to quantitatively decipher intra-plant N allocation and assimilation among plant species under no invasion and under the invasion of *Chromolaena odorata* and *Ageratina adenophora* in a tropical ecosystem of SW China. We found that the assimilation of N derived from both soil ammonium (NH_4^+) and nitrate (NO_3^-) were higher in leaves than in roots for invasive plants, leading to higher leaf N levels than native plants. Compared with the same species under no invasion, most native plants under invasion showed higher N concentrations and NH_4^+ assimilations in both leaves and roots, and increases in leaf N were higher than in root N for native plants under invasion. These results inform that preferential N allocation, dominated by NH_4^+-derived N, to leaves over roots as an important N-use strategy for plant invasion and co-existence in the studied tropical ecosystem.

1. Introduction

Exotic plant invasion has become an urgent environmental issue in past decades (Mack et al., 2000), which can be stimulated by soil

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nitrogen (N) loading (Liu et al., 2017) and has caused many negative impacts to the structure and functions of ecosystems (Dukes and Mooney, 1999; Liu et al., 2013a). Plant N use strategies are important for understanding the expansion of invasive plants and the co-existence of native plants in responses to environmental N loading (Bobbink et al., 2010). Plant N utilization mainly includes plant N acquisition and intra-plant N allocation and assimilation (Masclaux-Daubresse et al., 2010), which has important influences on the primary productivity and performance of plant species (Harrison et al., 2007; LeBauer and Treseder, 2008). To decipher intra-plant N allocation and assimilation can help to understand the mechanisms behind exotic plant invasion and native plants' co-existence or disappearance (Bazzaz and Grace, 1997; Feng et al., 2009).

Ammonium (NH_4^+) and nitrate (NO_3^-) in soils are important plant N sources (Britto and Kronzucker, 2002). Several methods have been used to describe the allocation and assimilation of soil N sources in plants. Earlier studies compared nitrate reductase activity (NRA) in leaves and roots to assess intra-plant NO_3^- allocation and reduction (Andrews, 1986). Higher NRA levels in leaves than in roots indicate more NO_3^- allocation to leaves than to roots (Stewart et al., 1992). Few studies analyzed inorganic N concentrations in xylem saps to assess the allocation of NO_3^- between leaves and roots and its linkages with plant growth (Cheeseman, 1993; Scheurwater et al., 2002). More recent studies employed ^{15}N-labeling methods to calculate the allocation of NH_4^+ and NO_3^- between leaves and roots (Kalcsits et al., 2015; Bueno et al., 2018). However, the plant uptake of field applied ^{15}N tracers was substantially influenced by microbes and differed between NH_4^+ and NO_3^-, so that it is difficult to obtain accurate allocating strategies of N sources in plants (Harrison et al., 2007; Bueno et al., 2018). The location of N reduction in plants is not necessarily the same location of the final N assimilation because reduced N can be further reallocated between leaves and roots before the final N assimilation (Schjoerring et al., 2002). Also, it remains highly uncertain whether and how much the ^{15}N supplied as NH_4^+ and NO_3^- would exist in their initial inorganic forms or have been assimilated into biomass organic N, because almost all tracer studies have measured only the total ^{15}N recovered from the leaves and roots, which cannot distinguish ^{15}N allocation from ^{15}N assimilation (Bueno et al., 2018). Accordingly, these methods did not provide direct evidence on the final allocation and assimilation of soil N sources between roots and leaves. Moreover, most of the existing methods and studies have been conducted on crop plants incubated with NO_3^- or NH_4^+, and much less on plants in invaded ecosystems and by both N forms simultaneously (Stewart et al., 1992; Scheurwater et al., 2002). Therefore, it is highly valuable to develop a new method to decipher the allocation and assimilation of soil NH_4^+ and NO_3^- sources between leaves and roots of terrestrial plants, which would open a new window to understand the intra-plant N allocation and assimilation strategies of different plant species in the 'real' world of invaded ecosystems.

Combining measurements of N concentration and natural N isotopes (expressed as $\delta^{15}N$ values) of leaves and roots can help decipher intra-plant N allocation in natural ecosystems (Kolb and Evans, 2002; Sardans et al., 2017). Positive correlations often exist between leaf N (N_{leaf}) and root N (N_{root}) concentrations, and the correlations differ among plant species and under different environmental conditions due to different intra-plant N allocation patterns (Kerkhoff et al., 2006; Yan et al., 2016; Zhang et al., 2018a). Higher slopes of leaf-root N correlations or higher ratios of leaf N to root N (N_{leaf}/N_{root}) indicate greater N allocation to leaves (Reich, 2002; Zhang et al., 2018a; Zhang et al., 2018b). This can happen to plants with relatively high N-uptake abilities or under high N availability, which can support and benefit above-ground photosynthesis and growth (Laungani and Knops, 2009; Fraterrigo et al., 2011). For plants with relatively low N-competing abilities or under low N availability, preferential and greater N allocation to roots can promote root growth and augment N acquisition, leading to relatively low N_{leaf}/N_{root} values (Bazzaz and Grace, 1997; Wright et al., 2004). However, the relative contributions of NH_4^+ and NO_3^- have not been further disentangled for N_{leaf} or N_{root}, which is limiting the application of N_{leaf}/N_{root} to elucidate intra-plant N allocation and assimilation (Kolb and Evans, 2002; Pardo et al., 2012). Currently, it is crucial to determine the contributions of NH_4^+ and NO_3^- to N_{leaf} and N_{root}, which would substantially improve the understanding of how different plants allocate and assimilate soil N sources between leaves and roots.

Leaf $\delta^{15}N$ ($\delta^{15}N_{leaf}$) has been broadly used to indicate soil N availability and the openness of the ecosystem N cycle (Craine et al., 2009; Yang et al., 2013). However, differences often exist between $\delta^{15}N_{leaf}$ and root $\delta^{15}N$ ($\delta^{15}N_{root}$) across climate gradients and species (Craine et al., 2005, 2015), which suggests that $\delta^{15}N_{leaf}$ cannot simply be interpreted as the $\delta^{15}N$ of whole-plants to reflect fractional contributions of soil N sources at whole-plant level (Pardo et al., 2012). It is essential to accurately understand the $\delta^{15}N$ differences related to N-use strategies between leaves and roots (Houlton et al., 2007; Liu et al., 2013b). Combining $\delta^{15}N_{leaf}$ with $\delta^{15}N_{root}$ can give more insights into the intra-plant allocation and assimilation of NH_4^+ and NO_3^- (Fig. 1, Peuke et al., 2013; Kalcsits et al., 2014). More specifically, the N uptake associated with mycorrhizae can make the $\delta^{15}N$ of plant-assimilated N lower than that of plant sources (Craine et al., 2009; Hobbie and H€ogberg, 2012). The NO_3^- entered into plants will be reduced by nitrate reductase to nitrite and then by nitrite reductase to NH_4^+, and the synthesis of amino acids from NH_4^+ will be assimilated into biomass N. This process can take place in roots and leaves (Masclaux-Daubresse et al., 2010). Both NO_3^-

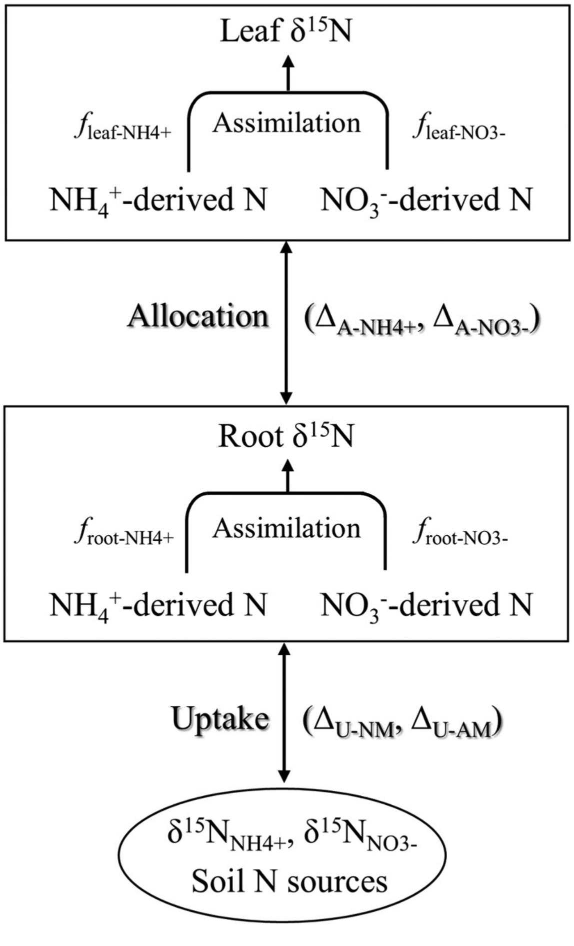

Fig. 1. Schematic isotope relationships between soil N sources and N in plant leaves and roots. The $\delta^{15}N_{NH4+}$ and $\delta^{15}N_{NO3-}$ are $\delta^{15}N$ values of soil NH_4^+ and NO_3^-, respectively. The NH_4^+-derived and NO_3^--derived N are leaf or root N derived from the assimilations of soil NH_4^+ and NO_3^-, respectively. Δ_{U-NM} and Δ_{U-AM} are isotope effects of N uptake for plants associated with no mycorrhizae and arbuscular mycorrhizae, respectively. Δ_{A-NH4+} and Δ_{A-NO3-} are isotope effects of NH_4^+-derived and NO_3^--derived N allocation between leaves and roots, respectively.

and NH_4^+ assimilation in plants discriminate against ^{15}N (Evans, 2001), thus unassimilated NO_3^- and NH_4^+ in plants have higher $\delta^{15}N$ values than their organic N products (Ledgard et al., 1985; Yoneyama et al., 1993; Peuke et al., 2013). Differing allocation of relatively ^{15}N-enriched N sources and relatively ^{15}N-depleted N sources between leaves and roots would cause differences between $\delta^{15}N_{leaf}$ and $\delta^{15}N_{root}$ values (Kolb and Evans, 2002; Pardo et al., 2012). When $\delta^{15}N$ values of plant N sources and isotope effects due to plant N uptake and intra-plant N allocation can be constrained, systematic observations of $\delta^{15}N_{leaf}$ and $\delta^{15}N_{root}$ values provide a new opportunity to evaluate contributions of NO_3^--derived and NH_4^+-derived N to N_{leaf} and N_{root}, respectively (Fig. 1). However, no study has established a quantitative framework to combine N concentrations and $\delta^{15}N$ values of soils, leaves, and roots to decipher intra-plant N allocation and assimilation in natural ecosystems.

Based on the above contexts, this work investigated N concentrations and N isotopes of soils, roots, and leaves among plant species under no invasion and the invasion of two exotic composite species in a tropical ecosystem of SW China. Our objectives were 1) to establish a new isotope framework to quantitatively decipher intra-plant N allocation and assimilation, 2) to explicitly dissect how intra-plant N allocation differ between invaders and natives, and between natives under no invasion and natives under invasion. Such a new method and new insights into intra-plant N allocation and assimilation can contribute substantially to the understanding of plant N-use strategies in terrestrial ecosystems.

2. Materials and methods

2.1. Study site

This study was conducted at Mt. Kongming (101.12°E, 22.14°N), Xishuangbanna, Yunnan province, SW China. Mt. Kongming is a montane ecosystem of tropical China, which has a subtropical monsoonal climate, and with mean annual temperature and mean annual precipitation of 21 °C and 1600 mm, respectively. The soil and bedrock are yellow soil and limestone rock, respectively. The *C. odorata* and *A. adenophora* are perennial native herbs in Mexico but noxious invasive forbs in China. Since entering mainland China from Xishuangbanna in the 1940s, these two invasive plants have caused many negative impacts on the terrestrial ecosystems in southwestern (SW) China (Feng et al., 2009). Native plants are mainly herbaceous and graminoid species that are widely distributed in tropical and subtropical China, with few deciduous and evergreen shrubs and occasional conifer forests (mainly *Pinus massoniana*). Details of the study site, experimental blocks and plots, and native plant species have been described in Hu et al. (2019). Briefly, there are obvious patches of *C. odorata* invasion (about 20 m × 20 m), *A. adenophora* invasion (about 20 m × 20 m), and no invasion (about 10 m × 10 m) in each block. Leaves and roots of the dominant species were sampled for each of three plots (2 m × 2 m for each) in no invasion patches of three blocks (one plot for each), six plots (2 m × 2 m for each) in *C. odorata* invasion patches of three blocks (two plots for each), and six plots (2 m × 2 m for each) in *A. adenophora* invasion patches of three blocks (two plots for each).

2.2. Sample collection and chemical analyses

In July of 2011, leaves and roots of invasive and dominant native plant species were sampled. Sampled native plants belonging to 36 species (8 shrub and 28 herb species) and 15 families (Mainly Poaceae, Asteraceae, Rubiaceae, Lamiaceae, Euphorbiaceae, etc) (Hu et al., 2019). In each plot, leaves and roots of the same individual for each plant species were simultaneously collected from 5 to 10 individuals and pooled together into one replicate sample of leaf and root, respectively. Thus, the replicate sample of each native species ranged from 1 to 3 under no invasion, and ranged from 1 to 6 under *C. odorata* or *A. adenophora* invasion based on the presence of the native species in each plot. Leaf N concentrations and $\delta^{15}N$ values have been reported in Hu et al. (2019), focusing on plant N uptake and preference under different invasive pressures. The present paper focuses on root-leaf N allocation by adding root N concentrations and $\delta^{15}N$ analyses.

Methods for measuring plant N concentrations and $\delta^{15}N$ values were identical to those reported in Hu et al. (2019). Briefly, leaf and root N concentrations (mg/g, dry weight (dw)) were determined using an elemental analyzer (Elementar Analysensysteme GmbH, Germany). Then, $\delta^{15}N$ values of N in each leaf and root sample (about 50 μg N) were determined by an isotope ratio mass spectrometer (Thermo MAT 253, Thermo Scientific, Bremen, Germany) coupled with an elemental analyzer (Flash EA 2000). The average standard deviations for replicate analyses of an individual sample were ±0.02% for N concentration and ±0.1‰ for $\delta^{15}N$ values.

2.3. Methods for estimating fractional contributions of soil NH_4^+ and NO_3^- to plant N

Most studies on plant N uptake in tropical ecosystems have stressed inorganic N due to its have higher N-cycle rates compared with high-latitude and high-altitude ecosystems where N availability are generally lower and plants rely more on organic N sources (Houlton et al., 2007; Liu et al., 2018; Zhang et al., 2018c). Moreover, plant dissolved organic nitrogen (DON) use was often demonstrated experimentally by the uptake of one or a few ^{15}N-labeled amino acids, which cannot elucidate the real availability of the whole soil DON pool to plants (Kahmen et al., 2009), so that substantial uncertainties existed in the source contributions estimated by using the $\delta^{15}N$ of the whole DON pool (e.g., Liu et al., 2013). Besides, the site is pristine with no agricultural or other human disturbance, and had lower bulk N deposition (<8 kg-N/ha/yr, Ackerman et al., 2016). Thus, the N source from N deposition has not been considered in this study. Consequently, soil NH_4^+ and NO_3^- were assumed as main plant N sources in this study (Fig. 1), and their respective fractional contributions to leaf N (denoted as $f_{\text{leaf-NH4+}}$ and $f_{\text{leaf-NO3-}}$ hereafter, respectively) and root N (hereafter as $f_{\text{root-NH4+}}$ and $f_{\text{root-NO3-}}$, respectively) were estimated by the following isotope mass-balance equations (Eqs. (1) & (2)).

$$\delta^{15}N_{leaf} + \Delta_U = \left(\delta^{15}N_{NH4+} - \Delta_{A-NH4+}\right) \times f_{leaf-NH4+} + \left(\delta^{15}N_{NO3-} - \Delta_{A-NO3-}\right) \times f_{leaf-NO3-} \tag{1}$$

$$\delta^{15}N_{root} + \Delta_U = \left(\delta^{15}N_{NH4+} + \Delta_{A-NH4+}\right) \times f_{root-NH4+} + \left(\delta^{15}N_{NO3-} + \Delta_{A-NO3-}\right) \times f_{root-NO3-} \tag{2}$$

where $f_{\text{leaf-NH4+}} + f_{\text{leaf-NO3-}} = 1$ and $f_{\text{root-NH4+}} + f_{\text{root-NO3-}} = 1$. $\delta^{15}N_{leaf}$, $\delta^{15}N_{root}$, $\delta^{15}N_{NH4+}$, and $\delta^{15}N_{NO3-}$ are $\delta^{15}N$ values of leaves, roots, soil NH_4^+, and soil NO_3^-, respectively, among which $\delta^{15}N_{NH4+}$ and $\delta^{15}N_{NO3-}$ values are cited from Hu et al. (2019). Δ_U values are isotope effects during plant N uptake (Fig. 1), which was 0.0‰ for plants with no mycorrhizal association and 2.0 ± 2.5‰ for plants associated with arbuscular mycorrhizae (Evans et al., 1996; Evans, 2001; Craine et al., 2009). $\Delta_{A\text{-}NO3\text{-}}$ and $\Delta_{A\text{-}NH4+}$ values are isotope effects of allocation of soil NO_3^--derived and NH_4^+-derived N between leaves and roots, respectively (Fig. 1). Previously, $\delta^{15}N_{root}$ values have been found lower by ca. 2.0 ± 1.9‰ and $\delta^{15}N_{leaf}$ values would be higher by ca. 2.0 ± 0.7‰ than $\delta^{15}N$ values of source NO_3^- due to the intra-plant allocation or redistribution of NO_3^--derived N (Kohl and Shearer, 1980; Mariotti et al., 1982; Bergersen et al., 1988; Yoneyama and Kaneko, 1989; Evans et al., 1996; Kalcsits et al., 2015). Accordingly, the mean $\Delta_{A\text{-}NO3\text{-}}$ value of 2.0 ± 1.9‰ and 2.0 ± 0.7‰ was considered in our calculations. $\delta^{15}N_{root}$ values have been found lower by ca. 0.0 ± 0.7‰ and $\delta^{15}N_{leaf}$ values would be higher by ca. 0.2 ± 0.8‰ than $\delta^{15}N$ values of source NH_4^+ due to the intra-plant allocation or redistribution of NH_4^+-

derived N (Yoneyama et al., 1991; Evans et al., 1996; Kalcsits et al., 2015). Accordingly, $\Delta_{A\text{-}NH4+}$ was considered as 0.0 ± 0.7‰ and 0.2 ± 0.8‰ in our calculations. Errors of f values in Eqs. (1) & (2) were estimated by a Monte Carlo method in each calculation.

Based on calculated $f_{leaf\text{-}NH4+}$, $f_{leaf\text{-}NO3-}$, $f_{root\text{-}NH4+}$, $f_{root\text{-}NO3-}$ values, contributions of soil NH_4^+ and NO_3^- to leaf N concentrations (hereafter as $N_{leaf\text{-}NH4+}$ and $N_{leaf\text{-}NO3-}$, respectively) and root N concentrations (hereafter as $N_{root\text{-}NH4+}$ and $N_{root\text{-}NO3-}$, respectively) were further calculated by the following equations (Eqs. (3)–(6)).

$$N_{leaf-NH4+} = N_{leaf} \times f_{leaf-NH4+} \tag{3}$$

$$N_{leaf-NO3-} = N_{leaf} \times f_{leaf-NO3-} \tag{4}$$

$$N_{root-NH4+} = N_{root} \times f_{root-NH4+} \tag{5}$$

$$N_{root-NO3-} = N_{root} \times f_{root-NO3-} \tag{6}$$

where N_{leaf} and N_{root} are total N of leaves and roots, respectively, f values were calculated by Eqs. (1) and (2).

2.4. Statistical analyses

Tukey HSD tests were used to identify significant differences in N concentrations and $\delta^{15}N$ values among different plant species. Before each analysis of significant differences, raw data were assessed for normality and homogeneity using the Shapiro-Wilks test and the Cochran's C-test, respectively. Transformations were performed when necessary to satisfy assumptions. Pearson correlation analyses were used to examine correlations between N_{leaf} and N_{root}, and $\delta^{15}N_{leaf}$ and $\delta^{15}N_{root}$. Statistical analyses were conducted using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The statistical significance was set at $p < 0.05$.

3. Results

3.1. Plant N concentrations

The N_{leaf} and N_{root} values averaged 23.6 ± 3.5 mg/g and 6.2 ± 2.2 mg/g for invasive plants and averaged 11.6 ± 5.3 mg/g and 6.2 ± 2.7 mg/g for native plants, respectively (Table 1, Fig. 2). Leaves had significantly higher N than roots, and they correlated positively for both invasive and native plants (Figs. 2, s1). Invasive plants had higher N_{leaf} than native plants, but N_{root} did not differ between them (Table 1), and therefore invasive plants showed higher N_{leaf}/N_{root} values than native plants (Table 1).

Compared the same species between invasion and no invasion plots, most native species under invasion increased both N_{leaf} and N_{root} (Figs. 3, s2), and increased more N in leaves than in roots (Fig. s2). Increasing N_{leaf} was found for 17 out of 19 native species under *C. odorata* invasion (ranged 0.0 mg/g to 9.8 mg/g and averaged 1.9 ± 2.5 mg/g), and for 14 out of 18 native species under *A. adenophora* invasion (ranged 0.4 mg/g to 10.7 mg/g and averaged 3.0 ± 2.9 mg/g) (Fig. 3). Increasing N_{root} was found for 12 out of 19 native species under *C. odorata* invasion (ranged 0.3 mg/g to 4.7 mg/g and averaged 0.4 ± 1.8 mg/g), and for 12 out of 18 native species under *A. adenophora* invasion (ranged 0.1 mg/g to 8.2 mg/g and averaged 0.6 ± 2.4 mg/g) (Fig. 3).

3.2. Plant $\delta^{15}N$ values

The $\delta^{15}N_{leaf}$ and $\delta^{15}N_{root}$ values averaged 0.6 ± 2.5% and −2.2 ± 1.6% for invasive plants, and averaged −0.8 ± 1.8% and −2.7 ± 1.8% for native plants, respectively (Table 1, Fig. 2). The $\delta^{15}N_{leaf}$ values were "therefore" higher than $\delta^{15}N_{root}$ values for both invasive and native plants (Table 1, Fig. 2). Invasive plants had higher $\delta^{15}N_{leaf}$ values than co-occurring native plants, but $\delta^{15}N_{root}$ values did not differ between them (Table 1). The $\delta^{15}N_{leaf}$ values were correlated positively with $\delta^{15}N_{root}$ for native plants under no invasion (Fig. s1), but such a relationship was not observed for invasive plants and native plants under invasion (Fig. s1).

3.3. Contributions of soil N source in leaves and roots

The $f_{leaf\text{-}NH4+}$ values averaged 63.4 ± 12.8% and 53.4 ± 9.9% for invasive and native plants, respectively (Table 2, Fig. s3). The $f_{root\text{-}NH4+}$ values averaged 27.5 ± 10.8% and 28.7 ± 13.5% for invasive and native plants, respectively, and were distinctly lower than corresponding $f_{root\text{-}NO3-}$ values (Table 2, Fig. s3).

Compared between invasive and native plants, both $N_{leaf\text{-}NH4+}$ and $N_{leaf\text{-}NO3-}$ in invasive plants were higher than those in native plants (Table 2, Fig. s3), while neither $N_{root\text{-}NH4+}$ nor $N_{root\text{-}NO3-}$ differed significantly between them (Table 2). Compared the same native species between invasion and no invasion, most native species increased $N_{leaf\text{-}NH4+}$ and $N_{root\text{-}NH4+}$ under both *C. odorata* and *A. adenophora* invasion, and decreased $N_{leaf\text{-}NO3-}$ and $N_{root\text{-}NO3-}$ under *C. odorata* invasion, increased $N_{leaf\text{-}NO3-}$ and $N_{root\text{-}NO3-}$ under *A. adenophora* invasion (Figs. 3, s2). $N_{leaf\text{-}NH4+}$ increased for 17 out of 19 native species under *C. odorata* invasion and for 13 out of 18 species under *A. adenophora* invasion (Figs. 3, s2). $N_{leaf\text{-}NO3-}$ decreased for 14 out of 19 species under *C. odorata* invasion and increased for 14 out of 18 species under *A. adenophora* invasion (Figs. 3, s2). $N_{root\text{-}NH4+}$ increased for 16 out of 19 species under *C. odorata* invasion and for 10 out of 18 species under *A. adenophora* invasion (Figs. 3, s2). $N_{root\text{-}NO3-}$ decreased for 12 out of 19 species under *C. odorata* invasion and increased for 11 out of 18 species under *A. adenophora* invasion (Figs. 3, s2).

4. Discussion

4.1. Leaf-root N allocation and assimilation between invasive and native plants

Compared with native plants, higher N_{leaf}/N_{root} values of invasive plants suggested more N allocation to leaves than to roots for invasive plants (Table 1). Mechanistically, more N allocation to leaves reflects the higher N demand of invasive plants to achieve higher rates of leaf photosynthesis, growth, production, and expansion (Wright et al.,

Table 1
The N_{leaf}, N_{root}, N_{leaf}/N_{root}, $\delta^{15}N_{leaf}$, and $\delta^{15}N_{root}$ values of *A. adenophora* ($n = 6$), *C. odorata* ($n = 6$), native plants under no invasion ($n = 59$), native plants under *A. adenophora* invasion ($n = 39$) and *C. odorata* invasion ($n = 45$) in Xishuangbanna, SW China. Values of mean ± SD (standard deviation) are shown. Different letters behind the mean values of the same row indicate significant differences at the level of $p < 0.05$.

Variables	No invasion	*A. adenophora* invasion		*C. odorata* invasion	
	Natives	*A. adenophora*	Natives	*C. odorata*	Natives
N_{leaf} (mg/g, dw)	$10.7^b \pm 4.7$	$22.7^a \pm 3.2$	$12.3^b \pm 5.6$	$24.6^a \pm 3.6$	$12.4^b \pm 5.6$
N_{root} (mg/g, dw)	$5.8^{ab} \pm 2.4$	$4.9^b \pm 1.3$	$5.9^{ab} \pm 2.4$	$7.6^a \pm 2.3$	$7.1^a \pm 3.3$
N_{leaf}/N_{root}	$2.0^b \pm 0.9$	$4.8^a \pm 0.9$	$2.3^b \pm 1.2$	$3.5^a \pm 0.7$	$2.1^b \pm 1.3$
$\delta^{15}N_{leaf}$/‰	$-1.4^b \pm 1.1$	$1.1^a \pm 2.8$	$-1.3^b \pm 1.3$	$0.2^{ab} \pm 2.1$	$-0.5^b \pm 1.8$
$\delta^{15}N_{root}$/‰	$-3.4^b \pm 1.3$	$-2.5^{ab} \pm 1.5$	$-3.0^{ab} \pm 1.7$	$-1.9^{ab} \pm 1.6$	$-1.4^a \pm 1.9$

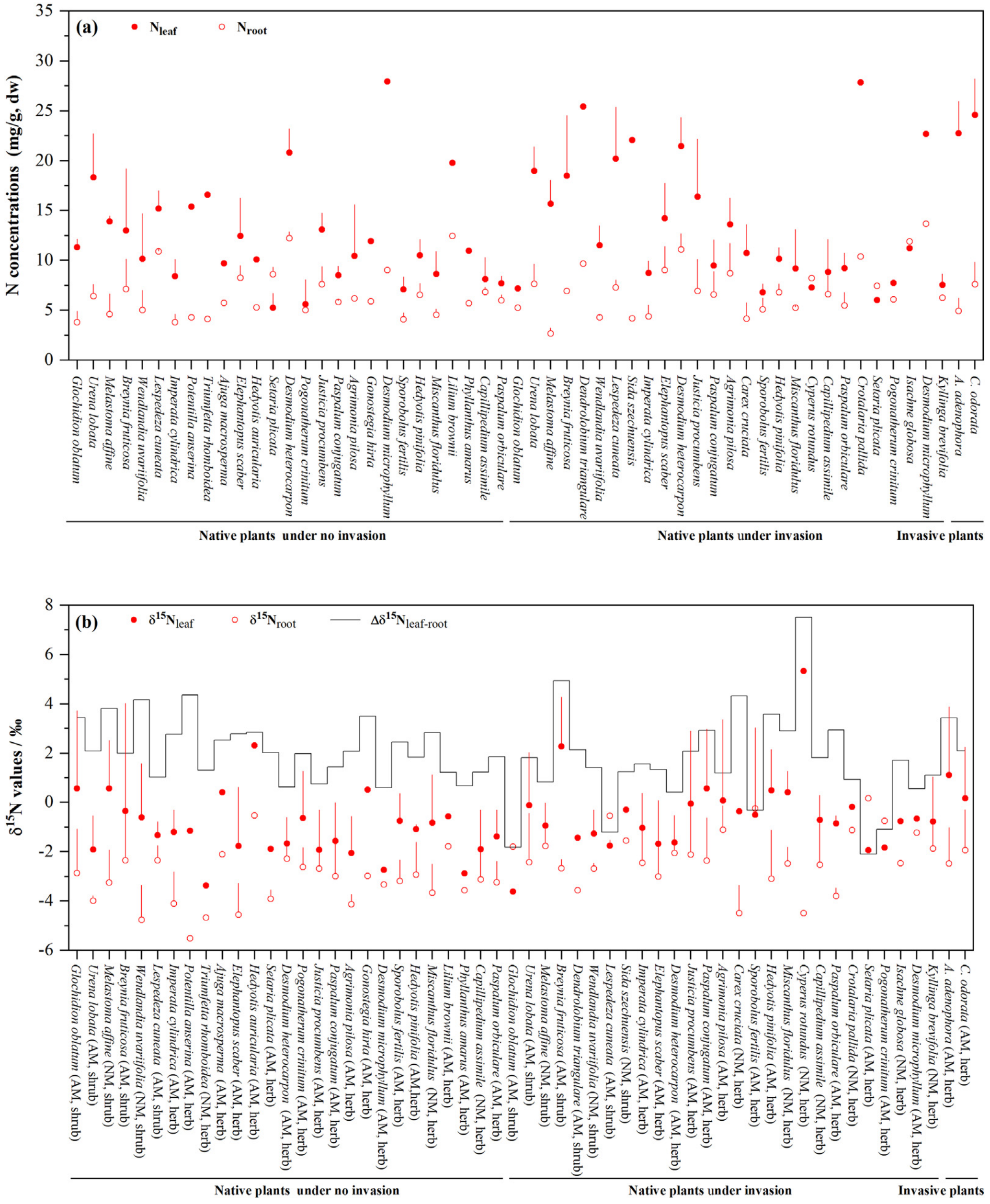

Fig. 2. N_{leaf} and N_{root} (a), $\delta^{15}N_{leaf}$, $\delta^{15}N_{root}$, and $\Delta\delta^{15}N_{leaf\text{-}root}$ values (b) of native plants under no invasion ($n = 1$–3 for each species), native plants under invasion ($n = 1$–12 for each species), and invasive plants ($n = 6$ for each species) in Xishuangbanna, SW China. Mean ± SD values are shown. In panel a, N_{leaf} and N_{root} are N concentrations of leaves and roots, respectively. In panel b, $\Delta\delta^{15}N_{leaf\text{-}root}$ values (the H line) were calculated using $\delta^{15}N_{leaf}$ minus $\delta^{15}N_{root}$ of the same species, AM, arbuscular mycorrhizae, and NM, non-mycorrhizal plants.

2004; Hu et al., 2019). These results provide clear evidence on the overall N allocation and assimilation strategy for invasive plants, which adds field evidence to the preferred N allocation to photosynthetic units found previously in incubated plants of *A. adenophora* (Feng et al., 2009).

Based on detailed results of isotope mass balance calculations (detailed in Methods), both $N_{leaf\text{-}NH4+}$ and $N_{leaf\text{-}NO3\text{-}}$ in invasive plants were higher than those in native plants (Table 2), while neither $N_{root\text{-}NH4+}$ nor $N_{root\text{-}NO3\text{-}}$ differed significantly between them (Table 2). These results suggest that the assimilation of N derived from both soil NH_4^+ and NO_3^- were higher in leaves than in roots for invasive plants, leading to higher N_{leaf} and N_{leaf}/N_{root} levels than in native plants. More N allocation to leaves than to roots can greatly increase the light-saturated photosynthetic rate and photosynthetic N use efficiency, and therefore plant biomass (Takashima et al., 2004). Previous studies observed that invasive plants often exhibit higher above-ground biomass than native plants, while below-ground biomass does not differ between them (te Beest et al., 2009; Fraterrigo et al., 2011). The strategy of N allocation observed in the studied invasive plants provides a mechanistic explanation for the higher above-ground biomass of invaders compared to native plants (Fraterrigo et al., 2011) and on the higher N competitiveness of invasive plants (Pattison et al., 1998).

4.2. Leaf-root N allocation and assimilation between native plants under invasion and no invasion

Compared with the same species under no invasion, most native plants under invasion had increased N in both leaves and roots (Fig. 3). This is evidence for increased N uptake of native plants under invasion (Laungani and Knops, 2009). Interestingly, most native plants under invasion showed increased N_{leaf}/N_{root} values, suggesting that they allocated more N to leaves than to roots relative to natives under

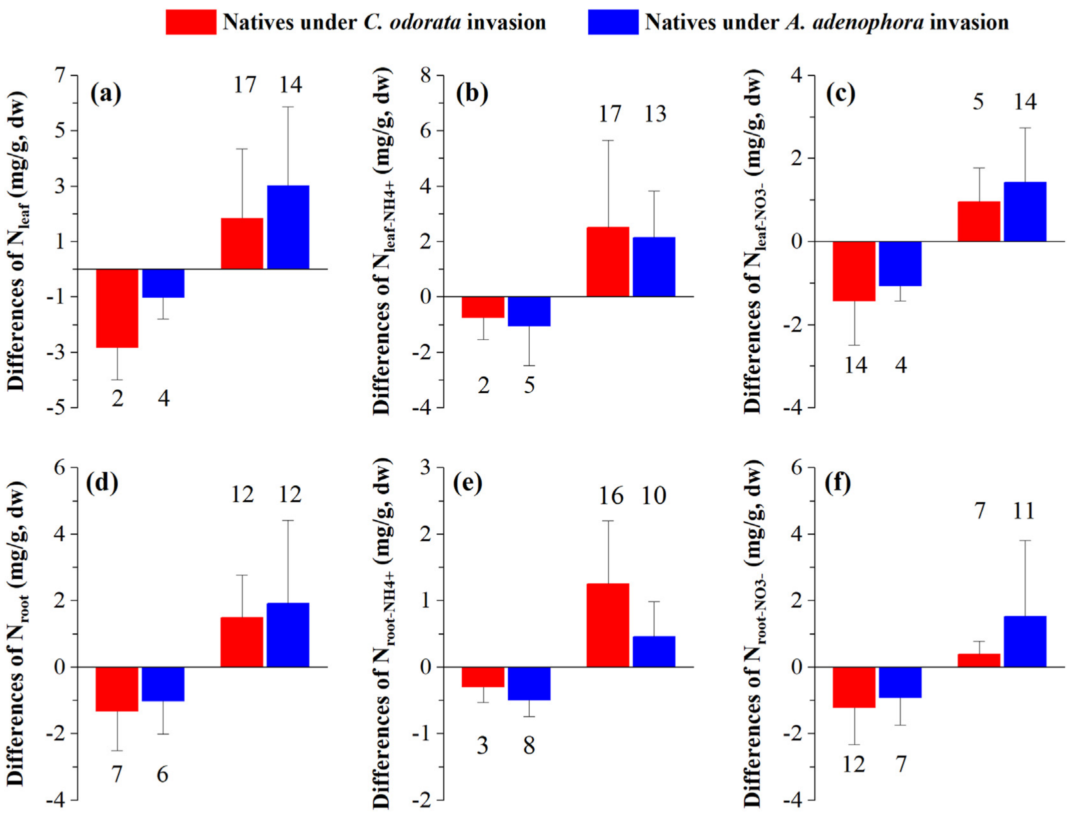

Fig. 3. Mean differences of N_{leaf} (a), $N_{leaf\text{-}NH4+}$ (b), $N_{leaf\text{-}NO3\text{-}}$ (c), N_{root} (d), $N_{root\text{-}NH4+}$ (e), and $N_{root\text{-}NO3\text{-}}$ (f) of native plants between invasion and no invasion. The difference values were calculated using values under invasion ($n = 1$–6) minus mean values of the same species under no invasion (detailed in Fig. S2). The bars and whiskers are mean and SD values, respectively. The positive and negative values denote increases and decreases in N concentrations for natives under invasion, respectively. The number above each column represents the species number with increases or decreases in N concentrations. $N_{leaf\text{-}NH4+}$, $N_{leaf\text{-}NO3\text{-}}$, $N_{root\text{-}NH4+}$, and $N_{root\text{-}NO3\text{-}}$ are leaf and root N concentrations contributed by soil NH_4^+ and NO_3^-, respectively.

no invasion (Fig. s2). This strategy resembled the N allocation strategy of invasive plants. In this study, soil N availability was generally high (Hu et al., 2019). Hence, more N allocation to photosynthetic organs occurred to support the growth of aboveground organs, the competitiveness of invasive, and the coexistence of native plants (Fortunel et al., 2012). This also explains why native plants under invasion could coexist and maintain similar above-ground biomass with those under no invasion (Feng et al., 2009). Conversely, Fraterrigo et al. (2011) observed that native plants under invasion allocated more N to roots than to leaves under low N supply, probably to promote root growth and simultaneously enhance the competition for soil N resources (Poorter et al., 2012). Increased N allocation to leaves of native plants under invasion occurred to adapt N availabilities and intensified N competition (Feng et al., 2009; Laungani and Knops, 2009).

Calculated results of soil NH_4^+ and NO_3^- contributions (detailed in Methods) further showed that $N_{leaf\text{-}NH4+}$ and $N_{root\text{-}NH4+}$ increased for most native plants under both *A. adenophora* and *C. odorata* invasion (Figs. 3, s2). However, $N_{leaf\text{-}NO3\text{-}}$ and $N_{root\text{-}NO3\text{-}}$ increased only for some natives under the *A. adenophora* invasion and even decreased for most natives under the *C. odorata* invasion (Figs. 3, s2). In general, our results therefore suggest that most of the native plants under invasion showed higher assimilation of N derived from soil NH_4^+ in both leaves and roots, which is the major reason for elevated N concentrations for native plants under invasion. Increased NH_4^+ assimilation of most native plants facilitated their coexistence (Rossiter-Rachor et al., 2009). Differently, their NO_3^- assimilation either increased to meet higher N demands under invasion or decreased because the enhanced NH_4^+ assimilation has already fulfilled increases in N demands (Bueno et al., 2018). Our results thereby provide new evidence for understanding plant co-occurrence in invaded ecosystems.

5. Conclusions

This work establishes a new framework using natural isotope methods to evaluate contributions of NH_4^+-derived and NO_3^--derived N to the total N assimilation in leaves and roots of plants, which can

Table 2

Estimated $f_{leaf\text{-}NH4+}$, $f_{root\text{-}NH4+}$, $N_{leaf\text{-}NH4+}$, $N_{leaf\text{-}NO3\text{-}}$, $N_{root\text{-}NH4+}$, and $N_{root\text{-}NO3\text{-}}$ of *A. adenophora* ($n = 6$), *C. odorata* ($n = 6$), native plants under no invasion ($n = 59$), and native plants under *A. adenophora* invasion ($n = 39$) and *C. odorata* invasion ($n = 45$) in Xishuangbanna, SW China. Values of mean ± SD (standard deviation) are shown. Different letters behind the mean values of the same row indicate significant differences at the level of $p < 0.05$.

Variables	No invasion	*A. adenophora* invasion		*C. odorata* invasion	
	Natives	*A. adenophora*	Natives	*C. odorata*	Natives
$f_{leaf\text{-}NH4+}$ (%)	51.2 ± 8.4	65.1 ± 14.6	51.7 ± 8.7	61.8 ± 10.8	58.1 ± 11.2
$f_{root\text{-}NH4+}$ (%)	25.5 ± 9.2	30.3 ± 10.3	22.7 ± 13.3	24.6 ± 10.7	38.8 ± 13.5
$N_{leaf\text{-}NH4+}$ (mg/g, dw)	5.4^b ± 2.4	15.0^a ± 4.7	6.4^b ± 3.0	15.3^a ± 4.8	7.4^b ± 4.1
$N_{leaf\text{-}NO3\text{-}}$ (mg/g, dw)	5.3^b ± 2.7	7.7^a ± 2.8	6.1^b ± 3.0	9.1^a ± 2.3	5.1^b ± 2.6
$N_{root\text{-}NH4+}$ (mg/g, dw)	1.6^b ± 1.0	1.6^b ± 0.8	1.4^b ± 1.1	1.9^b ± 0.8	2.8^a ± 1.5
$N_{root\text{-}NO3\text{-}}$ (mg/g, dw)	4.3^{ab} ± 1.6	3.4^b ± 0.8	4.5^{ab} ± 1.9	5.9^a ± 2.2	4.3^{ab} ± 2.3

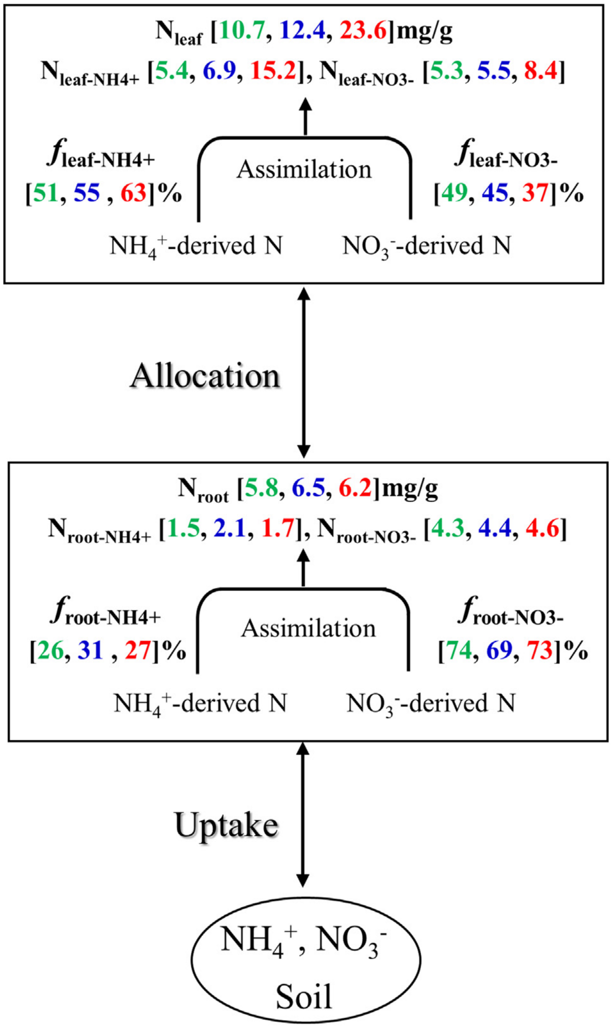

Fig. 4. Mean contributions of N derived from soil NH_4^+ and NO_3^- to total N in leaves and roots of different plants in an invaded ecosystem, SW China. Mean values of fractional contributions and N concentrations were calculated based on data in Tables 1 & 2 for all natives under no invasion (green fonts), all natives under invasion (blue fonts), and two invaders (red fonts).

be extended to broad terrestrial plants. Invasive plants allocated more NO_3^--derived and NH_4^+-derived N to leaves than native plants, while the N allocation to roots did not differ between invasive and native plants (Fig. 4). Most native plants under invasion increased the N assimilation in both leaves and roots, with more increases in leaves than in roots and in NH_4^+ than in NO_3^- assimilation (Fig. 4). Preferential N allocation, dominated by NH_4^+-derived N, to leaves over roots was revealed as a crucial N-use strategy for invasive plants and coexisting native plants, which might be an underlying mechanism for these plant species to optimize their N acquisition and to improve/maintain their productivity under invasion. In addition, invasive plants have more advantages in intra-plant N allocation than native plants, which would promote the expansion of the invasive plants in response to environmental N loading.

CRediT authorship contribution statement

X-Y. L and Y-B. L planned and designed the research, X-Y. L., Y-B. L and Y-H T conducted fieldwork, C-C.H. and X-Y. L. conducted experiments and performed data collection and analyses, wrote the manuscript; all authors commented on data interpretation and the whole manuscript.

Declaration of competing interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151203.

References

Ackerman, D., Millet, D.B., Chen, X., 2016. Global estimates of inorganic nitrogen deposition across four decades. Glob. Biogeochem. Cycles 33, 100–107.
Andrews, M., 1986. The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ. 9, 511–519.
Bazzaz, F.A., Grace, J., 1997. Plant Resource Allocation. Academic Press, San Diego, pp. 143–158.
Bergersen, F.J., Peoples, M.B., Turner, G.L., 1988. Isotopic discriminations during the accumulation of nitrogen by soybeans. Aust. J. Plant Physiol. 15, 407–420.
Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20, 30–59.
Britto, D.T., Kronzucker, H.J., 2002. NH4+ toxicity in higher plants: a critical review. J. Plant Physiol. 159, 567–584.
Bueno, A., Greenfield, L., Pritsch, K., Schmidt, S., Simon, J., 2018. Responses to competition for nitrogen between subtropical native tree seedlings and exotic grasses are species-specific and mediated by soil N availability. Tree Physiol. 39, 404–416.
Cheeseman, J.M., 1993. Plant growth modelling without integrating mechanisms. Plant Cell Environ. 16, 137–147.
Craine, J.M., Lee, W.G., Bond, W.J., Williams, R.J., Johnson, L.C., 2005. Environmental constraints on a global relationship among leaf and root traits of grasses. Ecology 86, 12–19.
Craine, J.M., Elmore, A.J., Aidar, M.P.M., Bustamante, M., Dawson, T.E., Hobbie, E.A., Kahmen, A., Mack, M.C., McLauchlan, K.K., Michelsen, A., et al., 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytol. 183, 980–992.
Craine, J.M., Brookshire, E.N.J., Cramer, M.D., Hasselquist, N.J., Kob, K., Marin-Spiotta, E., Wang, L.X., 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. Plant Soil 396, 1–26.
Dukes, J.S., Mooney, H.A., 1999. Does global change increase the success of biological invaders? Trends Ecol. Evol. 14, 135–139.
Evans, R.D., 2001. Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci. 6, 121–126.
Evans, R.D., Bloom, A.J., Sukrapanna, S.S., Ehleringer, J.R., 1996. Nitrogen isotope composition of tomato (Lycopersicon esculentum Mill. cv. T-5) grown under ammonium or nitrate nutrition. Plant Cell Environ. 19, 1317–1323.
Feng, Y.L., Lei, Y.B., Wang, R.F., Callaway, R.M., Valiente-Banuet, A., Inderjit, Li, Y.P., Zheng, Y.L., 2009. Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. Proc. Natl. Acad. Sci. USA 106, 1853–1856.
Fortunel, C., Fine, P.V., Baraloto, C., 2012. Leaf, stem and root tissue strategies across 758 neotropical tree species. Funct. Ecol. 26, 1153–1161.
Fraterrigo, F., Strickland, M.S., Keiser, A.D., Bradford, M.A., 2011. Nitrogen uptake and preference in a forest understory following invasion by an exotic grass. Oecologia 167, 781–791.
Harrison, K.A., Bol, R., Bardgett, R.D., 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. Ecology 88, 989–999.
Hobbie, E.A., H€ogberg, P., 2012. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New Phytol. 196, 367–382.
Houlton, B.Z., Sigman, D.M., Schuur, E.A.G., Hedin, L.O., 2007. A climate-driven switch in plant nitrogen acquisition within tropical forest communities. Proc.Natl. Acad.Sci. USA 104, 8902–8906.
Hu, C.C., Lei, Y.B., Tan, Y.H., Sun, X.C., Xu, H., Liu, C.Q., Liu, X.Y., 2019. Plant nitrogen and phosphorus utilization under invasive pressure in a montane ecosystem of tropical China. J. Ecol. 107, 372–386.
Kahmen, A., Livesley, S.J., Arndt, S.K., 2009. High potential, but low actual, glycine uptake of dominant plant species in three Australian land-use types with intermediate N availability. Plant Soil 325, 109–121.
Kalcsits, L.A., Buschhaus, H.A., Guy, R.D., 2014. Nitrogen isotope discrimination as an integrated measure of nitrogen fluxes, assimilation and allocation in plants. Physiol. Plant. 151, 293–304.

Kalcsits, L.A., Min, X.J., Guy, R.D., 2015. Interspecific variation in leaf–root differences in δ15N among three tree species grown with either nitrate or ammonium. Trees 29, 1069–1078.

Kerkhoff, A.J., Fagan, W.F., Elser, J.J., Enquist, B.J., 2006. Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. Am. Nat. 168, 103–122.

Kohl, D.H., Shearer, G., 1980. Isotope fractionation associated with symbiotic N2 fixation and uptake of NO3- by plants. Plant Physiol. 66, 51–56.

Kolb, K.J., Evans, R.D., 2002. Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. New Phytol. 156, 57–64.

Laungani, R., Knops, J.M.H., 2009. Species-driven changes in nitrogen cycling can provide a mechanism for plant invasions. Proc.Natl. Acad.Sci.USA 106, 12400–12405.

LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379.

Ledgard, S.F., Woo, K.C., Bergersen, F.J., 1985. Isotopic fractionation during reduction of nitrate and nitrite by extracts of spinach leaves. Aust. J. Plant Physiol. 12, 631–640.

Liu, X.Y., Koba, K., Makabe, A., Li, X.D., Yoh, M., Liu, C.Q., 2013b. Ammonium first: natural mosses prefer atmospheric ammonium but vary utilization of dissolved organic nitrogen depending on habitat and nitrogen deposition. New Phytol. 199, 407–419.

Liu, Y.J., Oduor, A.O., Zhang, Z., Manea, A., Tooth, I.M., Leishman, M.R., Xu, X., Kleunen, M.V., 2017. Do invasive alien plants benefit more from global environmental change than native plants? Glob. Chang. Biol. 23, 3363–3370.

Liu, X.Y., Koba, K., Koyama, L.A., Hobbie, S.E., Weiss, M.S., Inagaki, Y., Shaver, G.R., Giblin, A.E., Knute, S., Nadelhoffer, K.J., et al., 2018. Nitrate is an important nitrogen source for Arctic tundra plants. Proc.Natl. Acad.Sci.USA 115, 3398–3403.

Liu, X.J., Zhang, Y., Han, W.X., Tang, A.H., Shen, J.L., Cui, Z.L., Vitousek, P., Erisman, J.W., Guolding, K., Christie, P., Fangmeier, A., Zhang, F.S., 2013. Enhanced nitrogen deposition over China. Nature 494, 459–462.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecol.Appl. 10, 689–710.

Mariotti, A., Mariotti, F., Champigny, M.L., Amarger, N., Moyse, A., 1982. Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO3- by pearl Miller. Plant Physiol. 69, 880–884.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. 105, 1141–1157.

Pardo, L.H., Semaoune, P., Schaberg, P.G., Eagar, C., Sebilo, M., 2012. Patterns in δ15N in roots, stems, and leaves of sugar maple and American beech seedlings, saplings, and mature trees. Biogeochemistry 112, 275–291.

Pattison, R.R., Goldstein, G., Ares, A., 1998. Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. Oecologia 117, 449–459.

Peuke, A.D., Gessler, A., Tcherkez, G., 2013. Experimental evidence for diel δ15N-patterns in different tissues, xylem and phloem saps of castor bean (Ricinus communis L.). Plant Cell Environ. 36, 2219–2228.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193, 30–50.

Reich, P.B., 2002. In: Waise, Y., Eshel, A., Kafkafi, U. (Eds.), Plant Roots: The Hidden Half. Marcel Dekker, Inc, New York, pp. 205–220.

Rossiter-Rachor, N.A., Setterfield, S.A., Douglas, M.M., Hutley, L.B., Cook, G.D., 2009. Invasive Andropogon gayanus (gamba grass) is an ecosystem transformer of nitrogen relations in Australian savanna. Ecol. Appl. 19, 1546–1560.

Sardans, J., Grau, O., Chen, H.Y.H., Janssens, I.A., Ciais, P., Piao, S.L., Penuelas, J., 2017a. Changes in nutrient concentrations of leaves and roots in response to global change factors. Glob. Chang. Biol. 23, 3849–3856.

Scheurwater, I., Koren, M., Lambers, H., Atkin, O.K., 2002. The contribution of roots and shoots to whole plant nitrate reduction in fast- and slow-growing grass species. J. Exp. Bot. 53, 1635–1642.

Schjoerring, J.K., Husted, S., Mäck, G., Mattsson, M., 2002. The regulation of ammonium translocation in plants. J. Exp. Bot. 53, 883–890.

Stewart, G.R., Joly, C.A., Smirnoff, N., 1992. Partitioning of inorganic nitrogen assimilation between the roots and shoots of cerrado forest trees of contrasting plant communities of South East Brazil. Oecologia 91, 511–517.

Takashima, T., Hikosaka, K., Hirose, T., 2004. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous Quercus species. Plant Cell Environ. 27, 1047–1054.

te Beest, M., Stevens, N., Olff, H., van der Putten, W.H., 2009. Plant-soil feedback induces shifts in biomass allocation in the invasive plant Chromolaena odorata. J. Ecol. 97, 1281–1290.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., et al., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827.

Yan, Z.B., Li, P., Chen, Y.H., Han, W.X., Fang, J.Y., 2016. Nutrient allocation strategies of woody plants: an approach from the scaling of nitrogen and phosphorus between twig stems and leaves. Sci. Rep. https://doi.org/10.1038/srep20099.

Yang, Y.H., Ji, C.J., Robinson, D., Zhu, B., Fang, H.J., Shen, H.H., Fang, J.T., 2013. Vegetation and soil 15N natural abundance in alpine grasslands on the Tibetan Plateau: patterns and implications. Ecosystems 16, 1013–1024.

Yoneyama, T., Kaneko, A., 1989. Variations in the natural abundance of 15N in nitrogenous fractions of komatsuna plants supplied with nitrate. Plant Cell Physiol. 30, 957–962.

Yoneyama, T., Omaia, T., Nakata, S., Yazaki, J., 1991. Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia by plants. Plant Cell Physiol. 32, 1211–1217.

Yoneyama, T., Kamachi, K., Yamaya, T., Mae, T., 1993. Fractionation of nitrogen isotopes by glutamine synthetase isolated from spinach leaves. Plant Cell Physiol. 34, 489–491.

Zhang, J.H., He, N.P., Liu, C.C., Xu, L., Yu, Q., Yu, G.R., 2018a. Allocation strategies for nitrogen and phosphorus in forest plants. Oikos 127, 1506–1514.

Zhang, Q., Xiong, G.M., Li, J.X., Lu, Z.J., Li, Y.L., Xu, W.T., Wang, Y., Zhao, C.M., Tang, Z.Y., Xie, Z.Q., et al., 2018b. Nitrogen and phosphorus concentrations and allocation strategies among shrub organs: the effects of plant growth forms and nitrogen-fixation types. Plant Soil 427, 305–319.

Zhang, Z.L., Li, N., Xiao, J., Zhao, C.Z., Zou, C.Z., Zou, T.T., Li, D.D., Liu, Q., Yin, H.J., 2018c. Changes in plant nitrogen acquisition strategies during the restoration of spruce plantations on the eastern Tibetan Plateau, China. Soil Biol. Biochem. 119, 50–58.