

Relationships between plant nitrogen economy and life history in three deciduous-forest herbs

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Summary

1 We compared nitrogen (N) uptake and whole-plant N dynamics in three deciduous-forest herbs of contrasting life histories: the spring ephemeral *Allium tricoccum*, the summergreen *Viola pubescens* and the semievergreen *Tiarella cordifolia*. We predicted that differences in above-ground physiology would translate into differences in N acquisition and partitioning, such that nitrogen-use efficiency (NUE) would increase from *Allium* to *Viola* to *Tiarella*.

2 Patterns of N uptake were generally the opposite of our predictions. *Allium* had the lowest N uptake capacity in both laboratory and field experiments whereas roots of *Tiarella* had the highest specific N uptake capacity.

3 *Viola* was the only species in which the specific uptake capacity of roots was related to photosynthetic activity of leaves, both decreasing by a factor of two from spring to summer. In contrast, *Tiarella* consistently had the lowest photosynthetic capacity and the highest specific uptake capacity whereas *Allium* maintained substantial root uptake capacity throughout the summer when it had no photosynthetic activity.

4 There were no significant differences between species in overall NUE. However, there were differences in the components of NUE: nitrogen productivity (A) and mean residence time of N in the plant (MRT). Nitrogen productivity increased, and MRT decreased, from *Allium* to *Viola* to *Tiarella*.

5 In all three species, there was a balance between acquisition of N and building of biomass over the annual growth cycle, despite dramatic disjunctions between the tissue-specific rates of carbon and N acquisition in *Allium* and *Tiarella*. The variation in A and MRT we observed among co-occurring species of a single N-rich habitat was comparable with that observed by other researchers studying plants adapted to habitats of widely varying N availability.

Key-words: life history, nitrogen uptake, nitrogen-use efficiency, spring ephemeral, understorey herbs

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Introduction

Dramatic, but predictable, changes in light availability occur beneath the canopy of temperate deciduous forests through the seasons (Hicks & Chabot 1985). Herbaceous plants exhibit two distinct strategies for coping with the deep shade cast by the overstorey canopy in summer. Spring ephemeral herbs avoid shade stress by exploiting the narrow window of full sun and adequate temperatures prior to canopy closure whereas summergreen and evergreen species have adapted to tolerate the long summer shade period (Hicks &

Chabot 1985). Spring ephemeral herb abundance tends to be associated with high soil fertility (Al Mufti *et al.* 1977; Rogers 1982; Host & Pregitzer 1991), possibly because the rapid photosynthetic rates characteristic of this life history require an abundance of water and mineral nutrients (Grime 1979, 1994). In contrast, deciduous forests on infertile sites are expected to be dominated by summergreen and evergreen herbs (Grime 1994).

Spring ephemerals might therefore be expected to possess a suite of characteristics associated with plants from high-resource habitats (i.e. rapid growth rate, high physiological capacity for resource capture and low nutrient-use efficiency) whereas shade-tolerant herbs would show the opposite traits, typically associated with low-resource habitats (Grime 1979, 1994; Vitousek 1982; Schulze & Chapin 1987; Chapin 1991). Above-ground

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resource acquisition, reflected by photosynthetic capacity, is indeed higher in spring ephemerals (Sparling 1967; Taylor & Pearcy 1976; Rothstein 1999). Patterns of mineral nutrient capture should parallel those of photosynthetic capacity as these two processes are believed to be interdependent (Bloom *et al.* 1985; Campbell *et al.* 1991; Chapin 1991; Grime 1994). However, to the best of our knowledge, relationships between life history and the physiology of mineral nutrient capture by deciduous forest herbs have never been investigated.

The sugar maple-basswood–*Osmorhiza* ecosystem type of north-western lower Michigan, USA, is characterized by both the highest rates of N mineralization and nitrification in the region (Zak & Pregitzer 1990), and an abundant and diverse herbaceous plant community (Host & Pregitzer 1991). We investigated patterns of N use in three members of this community: *Allium tricoccum* Ait., a spring ephemeral; *Viola pubescens* Ait., a summergreen species; and *Tiarella cordifolia* L., a semievergreen (*sensu* Mahall & Bormann 1978). We therefore compared N-use among plant species adapted to temporal niches of variable light availability, while holding soil fertility constant, rather than among plant species adapted to habitats differing in soil fertility (as in Ingstad 1976; Aerts & Caluwe 1994; Vázquez de Aldana & Berendse 1997). We hypothesized that N uptake capacity would parallel differences in photosynthetic capacity among species (*Tiarella* < *Viola* < *Allium*), as well as the seasonal changes in photosynthetic capacity observed in *Viola* and *Tiarella* (Rothstein 1999). We also hypothesized that nitrogen-use efficiency (NUE; or the efficiency of biomass production with respect to nitrogen) would be inversely related to leaf N concentration and positively related to leaf longevity, and species would thus be ranked in the reverse order to N uptake. In particular, we expected that the components of NUE (*sensu* Berendse & Aerts 1987) would vary between species, with nitrogen productivity decreasing, and mean residence time increasing, from *Allium* to *Viola* to *Tiarella*.

Materials and methods

STUDY SITE, SPECIES AND SAMPLING PROTOCOL

Our study was conducted in a single stand of northern hardwood forest located in Wexford County, northern lower Michigan, USA (44°20' N, 86°00' W) classified by Host & Pregitzer (1991) as belonging to the sugar maple-basswood–*Osmorhiza* ecosystem type. Mahall & Bormann's (1978) phenological classification was used to assign herbaceous species as (i) spring ephemerals: leaf development occurs at or before snowmelt and leaf mortality occurs during expansion of the forest canopy; (ii) summer greens: leaf development occurs before expansion of the forest canopy and leaf mortality occurs prior to canopy leaf drop in the autumn; and (iii) semievergreens, as for (ii), but leaf

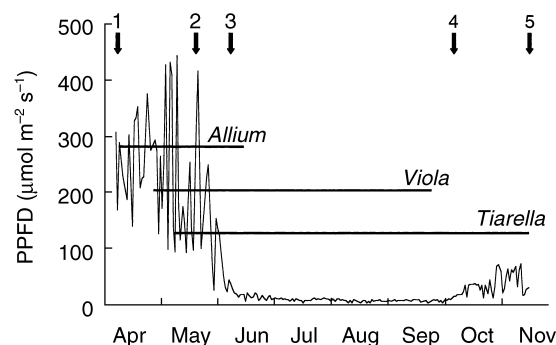


Fig. 1 Leaf phenology of *Allium tricoccum*, *Viola pubescens* and *Tiarella cordifolia* in relation to the average photosynthetic photon flux density (PPFD) reaching the forest floor each day of the snow-free year in 1997 (data from Rothstein 1999). Horizontal lines represent the time period during which each species has green leaves above ground. Numbered arrows at the top represent the timing of the following events: (1) snowmelt, (2) beginning of canopy bud-break, (3) canopy at full leaf expansion, (4) beginning of canopy leaf senescence, and (5) first snow accumulation.

mortality occurs over winter or early the following spring. We chose one spring ephemeral (*Allium tricoccum*), one summergreen (*Viola pubescens*) and one semievergreen (*Tiarella cordifolia*); nomenclature for all species follows Gleason & Cronquist 1991. In Fig. 1, we illustrate leaf phenology of the three study species in relation to canopy development and photosynthetically active radiation (PAR) reaching the forest floor.

Mitella diphylla, a closely related species that is difficult to distinguish from *Tiarella* when not flowering (Voss 1985), also occurs in this stand. It is therefore likely that some non-flowering individuals of *M. diphylla* were mistaken for *Tiarella*, but these two species also appear indistinguishable in terms of phenology, life history and selected parameters of leaf physiology and chemistry (Rothstein 1999).

Patterns of carbon (C) and N metabolism were determined through a series of physiological measurements conducted in both spring and summer, with a third sample for *Allium* and *Viola* in the late summer, and *Tiarella* in the autumn. Two sets of five and 10 ramets of each species were collected for measurement of CO₂ exchange and tissue N, and of specific root uptake of ¹⁵NH₄⁺ and ¹⁵NO₃⁻, respectively. For each set, a transect (random start point) across the stand was sampled at 20-m intervals, and the nearest plant (or for *Allium* and *Tiarella*, which reproduce clonally, the nearest solitary ramet) selected. Intact cylinders of soil, approximately 30 cm deep and 25 cm in diameter, containing each plant were placed in *c.* 10-L buckets for transport to the field laboratory (approximately 5 min).

PHOTOSYNTHESIS AND PLANT N

Light response curves were determined for the three species as part of a parallel study of their photosynthetic adaptations (Rothstein 1999), and used to estimate the instantaneous photosynthetic N-use

efficiency (PNUE). Upon completion of CO₂-exchange measurements, each plant was divided into component tissues (i.e. roots, stems, leaves), oven-dried to a constant weight at 70 °C, and then ground to a fine powder using a mortar and pestle. The ratio of root mass to total plant mass (i.e. root mass ratio; RMR) was calculated for each plant. The N content of each tissue sample was determined using an NC 2500 Elemental Analyser (CE Elantech Inc., Lakewood, NJ, USA). Because a substantial amount of leaf N can exist as NO₃⁻ (Chapin *et al.* 1988), we determined the NO₃⁻ content of each component tissue by extracting a *c.* 50-mg subsample in 5 mL of 100 °C deionized water, then analysed the extractant colourimetrically on a Rapid Flow Analyser (Alpkem Inc., Clackamas, OR, USA).

Because spring was the only time in which all three species displayed leaves, we only compared PNUE for this season. Springtime photosynthetic rate was estimated by entering a representative midday photon flux density (700 μmol m⁻² s⁻¹; 10-day average) into the light response function for each individual. Leaf-level PNUE was calculated for each plant, as the rate of CO₂ assimilation per unit of leaf N (μmol CO₂ g N⁻¹ s⁻¹).

¹⁵N UPTAKE

Ten plants of each species were washed free of soil particles in tap water, and then their root systems suspended in glass jars containing 750 mL of either 300 μmol L⁻¹ K¹⁵NO₃ (*n* = 5) or 300 μmol L⁻¹ ¹⁵NH₄Cl (*n* = 5) in 0.5 mmol L⁻¹ CaCl (to preserve membrane integrity; Epstein *et al.* 1963). These N concentrations were chosen to be high enough that uptake rates would not be limited by substrate concentration (Lewis 1986), but low enough to be within the range of soil solution concentrations observed in the field (Rothstein 1999). The plants were incubated at room temperature (18–21 °C) in the field laboratory for 1 h, after which the root system of each plant was washed in rapidly flowing tap water for *c.* 5 min. After drying, separation into component tissues and grinding to a fine powder, the ¹⁵N content of each tissue was determined by mass spectrometry (Stable Isotope Facility, Department of Agronomy and Range Science, University of California, Davis, CA, USA). The amount of ¹⁵N label within each plant tissue was calculated as the atom percentage ¹⁵N in excess of the background atom percentage ¹⁵N (determined on unlabelled plant samples). The amount of isotope within each tissue was summed to calculate whole-plant ¹⁵N. We then calculated specific uptake rate (μmol N g root⁻¹ h⁻¹) by dividing whole-plant ¹⁵N by the dry biomass of roots.

N RESORPTION

In order to compare patterns of N resorption, we collected shed leaves from five ramets each of *Allium* (June 1997) and *Tiarella* (April 1998). Nitrogen concentrations were analysed as described earlier and compared with green-leaf values (spring and summer,

respectively, for the two species). *Allium* appeared to undergo active leaf senescence, as evidenced by a clear pattern of basipetal yellowing whereas leaf loss in *Tiarella* appeared to be due to general necrosis, and/or mechanical damage. *Tiarella* leaves initiated in the spring of 1997 suffered 30% mortality by November of that year, 80% by snowmelt in the spring of 1998, and 100% within 1 month of snowmelt (Rothstein 1999). We could find no intact, shed leaves of *Viola* suggesting that this species loses its leaves primarily through mechanical damage and perhaps herbivory.

WHOLE-PLANT N DYNAMICS

In order to characterize patterns of *in situ* plant growth, N uptake, NUE and N partitioning, approximately 75 ramets of each species with intact roots were collected and labelled with ¹⁵N before return to the field for 1 full year of growth. Plants were collected when their leaves began to emerge from the soil in the spring of 1997 (6–10 April for *Allium*, 23–26 April for *Viola* and 10–13 May for *Tiarella*), assigned an identifying number, washed free of soil particles with tap water, blotted dry and then weighed. The root system of each plant was then placed for *c.* 2 h in a beaker containing a labelling solution of 500 μmol L⁻¹ each of ¹⁵NH₄Cl, K¹⁵NO₃, in 0.5 mmol L⁻¹ CaCl₂. Roots were then washed in rapidly flowing tap water for *c.* 5 min. A randomly selected subsample of 15 plants from each species (hereafter referred to as 'initial' plants) was harvested immediately. The remaining plants were placed in 10-cm diameter by 15-cm deep PVC sleeves, sealed at the bottom with nylon screen (*c.* 1 mm openings), which were then filled with homogenized native soil and placed back in the ground in the original stand. Harvests of each species (10 randomly selected plants per harvest) were made throughout the next year to coincide with important phenological events (e.g. top senescence for *Allium*, canopy leaf senescence for *Tiarella*). *Tiarella* experienced significant mortality (*c.* 33%) in June and July, and the three subsequent harvests of this species were therefore of only five plants each. There was very little mortality of *Viola* (*c.* 3%) or *Allium* (*c.* 10%) throughout the course of the experiment, and no other apparent effects of transplanting; in particular, none of the containers appeared root bound, even at final harvest.

At each harvest, dry weight, total N and ¹⁵N were determined as described previously. The initial harvest of 15 plants for each species was used to determine the relationships between fresh and dry weight, and between fresh weight and total N and ¹⁵N contents. These relationships were used to estimate initial values for the remaining (transplanted) individuals.

Allium

$$DW = (0.1891 \times FW) - 0.1641, r^2 = 0.968;$$

$$N = (0.006 \times FW) - 0.0001, r^2 = 0.992;$$

$$^{15}N = (10.589 \times FW) - 2.654, r^2 = 0.843.$$

Viola

$$\begin{aligned} DW &= (0.1878 \times FW) + 0.0032, r^2 = 0.922; \\ N &= (0.006927 \times FW) - (0.00049 \times DAY) + 0.001402, \\ r^2 &= 0.890; \\ {}^{15}\text{N} &= (32.333 \times FW) - (3.991 \times DAY) + 3.426, \\ r^2 &= 0.879. \end{aligned}$$

Tiarella

$$\begin{aligned} DW &= (0.2068 \times FW) - 0.118, r^2 = 0.846; \\ N &= (0.004954 \times FW) + (0.000631 \times DAY) - 0.00245; \\ r^2 &= 0.805; \\ {}^{15}\text{N} &= (36.675 \times FW) - 3.986; r^2 = 0.847. \end{aligned}$$

Where DW is the initial plant dry weight (g), FW, the initial plant fresh weight (g), N, the initial N content of the plant (g), ${}^{15}\text{N}$, the initial ${}^{15}\text{N}$ content of the plant (μg), and DAY the day on which the plant was initially collected (1–4). Collection day was included as a coefficient in regression equations for *Viola* and *Tiarella*, where it improved the coefficient of determination for each. This effect was likely due to changing root:shoot ratios, because rapid leaf expansion was occurring over the 4 days it took to process all the plants.

Relative biomass growth (g g^{-1}) for each subsequently harvested plant was calculated by subtracting the estimate of its initial dry weight from its final dry weight and dividing by the estimate of initial dry weight, and relative N uptake (mg N g^{-1}) as final N minus initial N divided by the initial dry weight.

Patterns of N partitioning were followed by determining the amount of ${}^{15}\text{N}$ label within each plant tissue at each harvest. Atom percentage ${}^{15}\text{N}$ in excess of the background atom percentage ${}^{15}\text{N}$ (see ${}^{15}\text{N}$ uptake assay) was multiplied by the total N in that tissue to give a value in μg of ${}^{15}\text{N}$ in that tissue per plant, and then adjusted for differences in plant size by dividing by initial dry weight.

We used these same plants to determine NUE as defined by Berendse & Aerts (1987), i.e. the product of nitrogen productivity (A; or dry matter production per unit of plant nitrogen) and mean residence time (MRT) of nitrogen in the plant. The equations of Vázquez de Aldana & Berendse (1997) were used to estimate N productivity for the entire year of growth (A) and for the initial spring light phase (A_i):

$$A = \frac{B_{\text{final}} - B_{\text{initial}}}{\frac{N_{\text{final}} + N_{\text{initial}}}{2}} \quad \text{eqn 1}$$

and MRT:

$$\text{MRT} = \frac{1}{\frac{{}^{15}\text{N}_{\text{initial}} - {}^{15}\text{N}_{\text{final}}}{{}^{15}\text{N}_{\text{initial}}}} \quad \text{eqn 2}$$

where B is plant biomass, N is total plant nitrogen content and ${}^{15}\text{N}$ is the total amount of labelled nitrogen in the plant.

STATISTICAL ANALYSIS

Leaf N concentration, leaf NO_3^- concentration, ${}^{15}\text{N}$ uptake and RMR were analysed using one-way analysis of variance (ANOVA) with species–season combination as a single factor (either seven or nine levels); differences in mean values were evaluated using Tukey's HSD test. For whole-plant relative growth and relative N uptake, we compared only the final (i.e. after 1 year) values for each species using Bonferroni's multiple comparison procedure, with probabilities scaled for three pairwise comparisons. We used this same procedure to test for differences in PNUE, A, MRT and total NUE. Student's *t*-tests were used to compare N concentrations between green and shed leaves of *Allium* and *Tiarella*. Tissue NO_3^- concentrations, NO_3^- and NH_4^+ uptake rates and leaf NRA were log-transformed prior to statistical analyses to meet the assumption of normality. All statistical analyses were performed using SYSTAT for personal computers (Wilkinson 1990), and significance was accepted at $\alpha = 0.05$.

Results

There were significant differences among species and seasons in both total leaf N and leaf NO_3^- concentration (Fig. 2). Spring-time leaf N concentrations in *Allium* and *Viola* were approximately 70% greater than those of *Tiarella* (Fig. 2a), but while leaf N dropped dramatically in *Viola* from spring to summer, there were no seasonal changes in *Tiarella*. A substantial portion of the N in leaves of *Viola* and *Tiarella* was in the form of NO_3^- (1–16%), but NO_3^- was barely detectable in the leaves of *Allium* (Fig. 2b). Differences in leaf N concentration paralleled differences in photosynthetic rates (Rothstein 1999), such that there were no differences among species in springtime PNUE (Table 1; $P = 0.653$).

The roots of all three species took up less ${}^{15}\text{NO}_3^-$ (Fig. 3a) than ${}^{15}\text{NH}_4^+$ (Fig. 3b), although their relative preferences for NH_4^+ (i.e. the difference between rates of NH_4^+ and NO_3^- uptake) diminished from *Allium* to *Viola* to *Tiarella*. Nitrate and NH_4^+ uptake both followed the same pattern among species, decreasing from *Tiarella* to *Viola* to *Allium*. Specific uptake capacity of both ions tended to decrease over time for all species, although these changes were, for the most part, not statistically significant.

In situ N uptake by transplants of *Viola* and *Tiarella* (Fig. 4a) closely mirrored biomass accumulation (Fig. 4b). *Allium*, however, accumulated little N in the spring despite a very rapid relative growth rate, and even maintained net N uptake in the summer, despite a decline in biomass during this period. The negative relative N uptake in June was probably the result of N loss during leaf senescence.

Despite the fact that *Tiarella* roots had the greatest specific uptake capacity for NH_4^+ and NO_3^- (Fig. 3), *Viola* exhibited the most rapid rate of relative N uptake

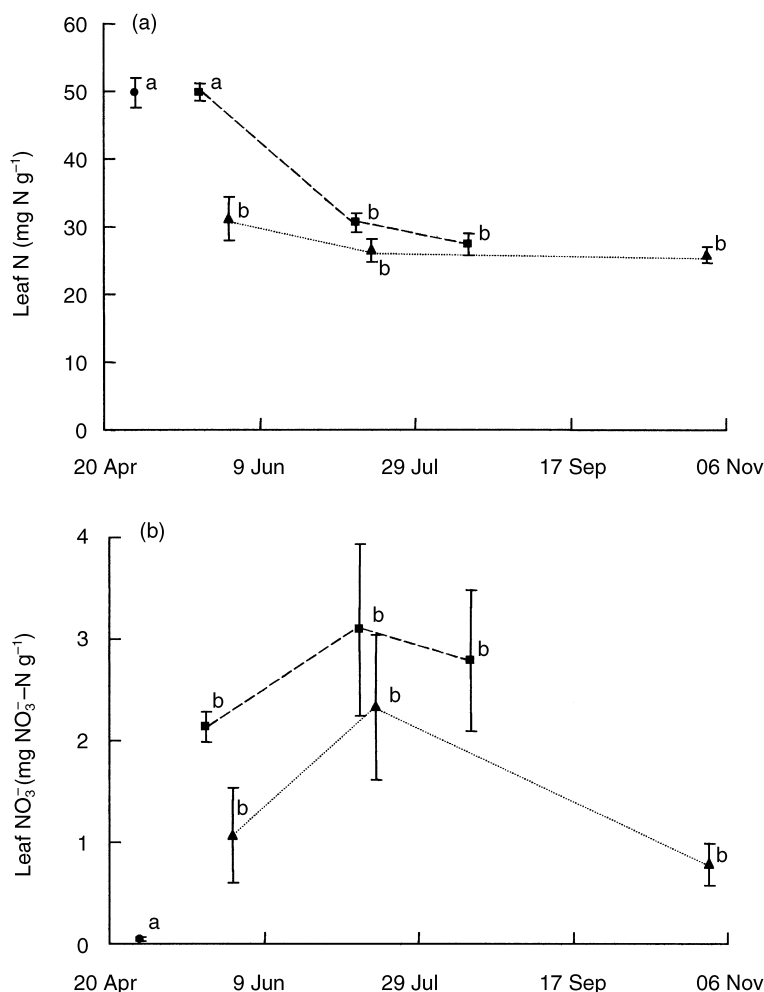


Fig. 2 Seasonal and interspecific patterns of (a) leaf N and (b) leaf NO_3^- concentration in *Allium tricoccum* (●), *Viola pubescens* (■) and *Tiarella cordifolia* (▲). *Allium* data are confined to the spring because of its limited leaf display. Values represent means (± 1 SE) of five ramets of each species. Means within a panel with the same letter are not significantly different according to Tukey's HSD test ($P > 0.05$).

Table 1 Components of nitrogen-use efficiency in *Allium*, *Viola* and *Tiarella*: springtime photosynthetic nitrogen-use efficiency (PNUE_{SPR}), springtime nitrogen productivity (A_{SPR}), annual nitrogen productivity (A), mean residence time of nitrogen (MRT), and nitrogen-use efficiency (NUE). Values are means ± 1 SE. Means in the same column followed by the same letter are not significantly different according to Bonferroni's multiple comparison test

Species	PNUE_{SPR} ($\mu\text{mol CO}_2 \text{ g N}^{-1} \text{ s}^{-1}$)	A_{SPR} ($\text{g biomass g}^{-1} \text{ N day}^{-1}$)	A ($\text{g biomass g}^{-1} \text{ N day}^{-1}$)	MRT (years)	NUE ($\text{g biomass g N}^{-1}$)
<i>Allium</i>	8.6 ± 0.2^a	0.98 ± 0.16^a	12.4 ± 2.1^a	2.84 ± 0.36^a	37.6 ± 8.8^a
<i>Viola</i>	7.8 ± 0.5^a	0.42 ± 0.05^b	19.4 ± 2.5^a	$2.05 \pm 0.21^{a,b}$	42.3 ± 8.3^a
<i>Tiarella</i>	8.8 ± 1.2^a	1.09 ± 0.11^a	31.2 ± 3.7^b	1.69 ± 0.21^b	55.9 ± 9.2^a

in situ (Fig. 4). This is probably due to the higher proportion of *Viola*'s total biomass in roots (averaged across all seasons, root mass ratio was significantly greater in *Viola* [0.46 ± 0.04] than in *Allium* [0.24 ± 0.02] or *Tiarella* [0.21 ± 0.04]). After 1 full year of growth, net relative biomass gain was significantly greater in *Tiarella* compared with *Allium* ($P = 0.032$), while *Viola* was intermediate, and not significantly different from either. Relative N uptake by *Allium* was significantly lower than that of *Viola* ($P = 0.002$), and marginally lower than that of *Tiarella* ($P = 0.072$). Values for the latter two species were equivalent.

These seasonal patterns of growth and N uptake gave rise to differences among the three species in the components of NUE (Table 1). *Viola* had a significantly lower springtime N productivity than the other two species ($P = 0.001$), although PNUE did not vary at this time. Over an entire year of growth, however, N productivity increased significantly from *Allium* to *Viola* to *Tiarella* ($P = 0.001$). Mean residence time of N followed the opposite pattern decreasing from *Allium* to *Viola* to *Tiarella* ($P = 0.023$). Therefore total NUE, the product of A and MRT, did not differ between species ($P = 0.388$).

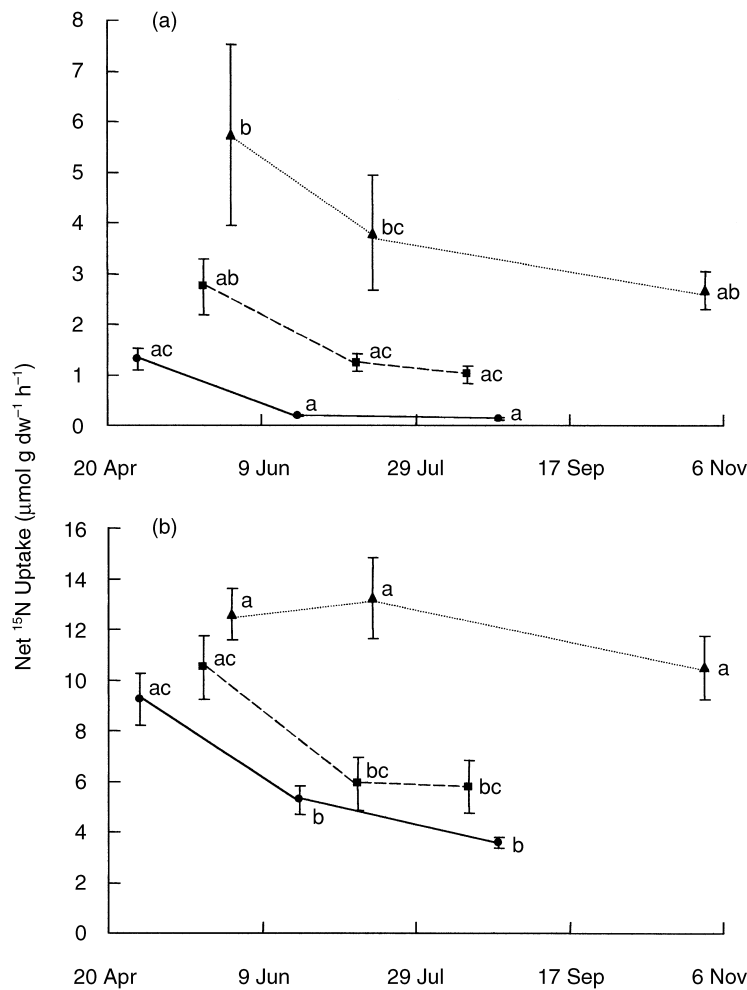


Fig. 3 Seasonal and interspecific patterns of (a) $^{15}\text{NO}_3^-$ and (b) $^{15}\text{NH}_4^+$ uptake. Values represent means (± 1 SE) of five ramets of each species. Means within a panel with the same letter are not significantly different according to Tukey's HSD test ($P > 0.05$). Symbols are as follows: *Allium tricoccum* (●), *Viola pubescens* (■) and *Tiarella cordifolia* (▲).

Both *Allium* and *Viola* showed seasonal shifts in N allocation associated with leaf mortality, with N moving from shoot to storage pools (Fig. 5). In contrast, shoots remain the dominant pool of N in *Tiarella* throughout the year. In the spring, when all three species had green leaves and there was direct light reaching the forest floor, *Allium* (0.69 ± 0.01) and *Tiarella* (0.62 ± 0.02) allocated proportionately more N to their shoots than did *Viola* (0.50 ± 0.03 ; $P < 0.001$).

Patterns of ^{15}N partitioning in the same plants (Table 2) are consistent with patterns of total N allocation. In all three species, ^{15}N that was in roots immediately following labelling had moved to leaves by the second harvest. After this, ^{15}N in *Allium* remained as a discrete pool (significantly the largest; Table 2) whereas the isotope dispersed rapidly throughout the entire plant in the other species so that no tissue pool could be distinguished statistically from any of the others after September 1997. After leaf senescence in *Allium*, the bulk of the label was translocated back to the bulb, which is consistent with the apparent resorption of 70% of green-leaf N in free-growing *Allium* plants (Table 3), and the increase in total N in the storage pool over this same period (Fig. 5). The relatively low

resorption observed in *Tiarella* (Table 3) is consistent with leaves remaining the largest pool of isotope, even if not significantly so (Table 2). *Viola* roots always contained a relatively large ^{15}N pool, although not always the largest and not significantly so after the initial harvest.

Discussion

We initially proposed that interdependence of above- and below-ground resource capture would lead to N uptake capacity and photosynthetic activity varying in parallel, both within and among species. However, we found that the species showed opposite rankings for rates of N uptake and photosynthesis, and patterns within species were inconsistent. We also proposed that NUE should increase with decreasing leaf N concentrations and increasing leaf longevity, but overall NUE did not differ among species. Moreover, its components (A and MRT) differed in the opposite direction to our predictions. Above- and below-ground physiology can therefore be uncoupled, and a variety of combinations of assimilation rates, allocation patterns and tissue phenologies used to balance above- and below-ground resource capture. Patterns of N uptake

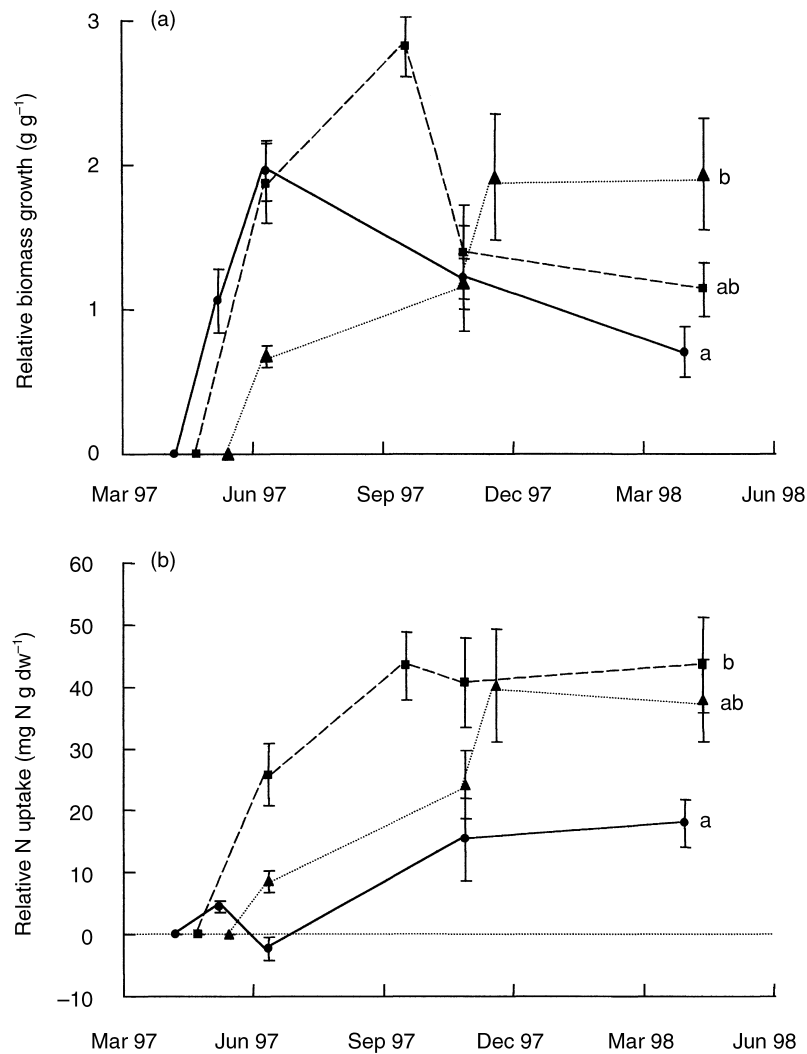


Fig. 4 (a) Relative biomass growth and (b) relative N uptake of *Allium*, *Viola* and *Tiarella* through 1 full year. Values represent means (± 1 SE) of 10 plants, except for the final three samples of *T. cordifolia*, which were of five plants each. Final growth and N uptake values with the same letter are not significantly different according to Bonferroni pairwise comparisons. Symbols are as follows: *Allium tricoccum* (●), *Viola pubescens* (■) and *Tiarella cordifolia* (▲).

and NUE also varied widely in response to ecological and life-history factors unrelated to N availability.

Species rank for photosynthetic capacity (*Tiarella* < *Viola* < *Allium*; Rothstein 1999) was the reverse of their rank for specific N uptake capacity (Fig. 3). Within species, *Viola* alone exhibited the predicted parallel response, with both photosynthetic capacity (Rothstein 1999) and N uptake capacity (Fig. 3) declining by a factor of two from spring to summer. Specific N uptake in *Tiarella* did not change from one season to another whereas its photosynthetic capacity decreased from spring to summer and then increased in autumn (Rothstein 1999). Perhaps the most unusual relationship between N uptake and photosynthesis was in *Allium*, which, despite the fact that it was leafless, sustained its NH_4^+ uptake capacity throughout the summer. Above- and below-ground resource capture, in terms of instantaneous physiological activity, are clearly not interdependent in *Allium* and *Tiarella*.

These disjunctions disappear for *Tiarella* when N uptake is measured *in situ*, with N uptake closely

tracking biomass growth throughout the year (Fig. 4). The disjunction was, however, reinforced by *in situ* measurements on *Allium*, where net uptake of N continued over the summer months despite a loss of biomass. Clearly, the rapid photosynthetic rates necessary to sustain the spring ephemeral life history (Sparling 1967; Taylor & Pearcy 1976; Rothstein 1999) require a large investment of N. However, *Allium* meets this demand from rapid remobilization of N from its bulb, accumulated as a result of uptake throughout the year, rather than by rapid springtime uptake. Indeed, even though *Allium* shows efficient resorption of N, spring uptake of N is outweighed by losses of N due to leaf senescence. However, in other spring ephemeral species, root senescence coincides with leaf senescence (e.g. *Erythronium americanum*; D. Rothstein personal observation), and sufficient N uptake must therefore occur in the spring.

Predictions of interdependence are based on the premise that, to optimize growth, plants must maintain a balance between acquisition of above- and below-ground

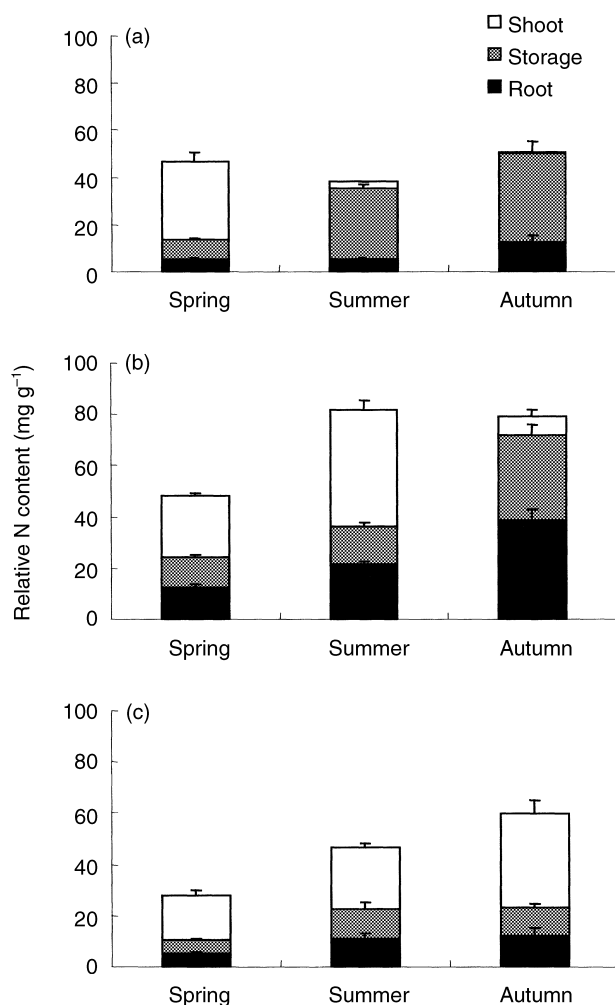


Fig. 5 Seasonal patterns of N allocation to shoots (leaves, stems and flowers), roots and storage (bulbs or rhizomes) in (a) *Allium tricoccum*, (b) *Viola pubescens*, and (c) *Tiarella cordifolia*. Values represent means (± 1 SE) of 10 ramets of each species, except for *Tiarella* in the autumn, in which means are of five ramets. All N values are expressed relative to the initial biomass of each plant.

Table 2 Changes in the distribution of ¹⁵N label in *Allium*, *Viola* and *Tiarella* through 1 year of growth. Values are means (± 1 SE) of the ¹⁵N label in each tissue, adjusted for initial plant size ($\mu\text{g } ^{15}\text{N g}^{-1}$ initial dry weight). Shoot includes leaves, stems and reproductive tissues. Storage includes bulbs and rhizomes. Values in bold type are significantly greater than all non-bold values within that sample date, using Tukey's HSD multiple comparison procedure ($P \leq 0.05$)

Distribution of ¹⁵ N label ($\mu\text{g } ^{15}\text{N g}^{-1}$ initial dry weight)							
	April 1997	May 1997	June 1997	September 1997	October 1997	November 1997	April 1998
<i>Allium</i>							
Shoot	0.5 \pm 0.11	83.9 \pm 12.59	6.38 \pm 1.14*	–	–	–	8.5 \pm 1.18
Storage	7.1 \pm 0.77	13.8 \pm 1.63	68.5 \pm 7.21	–	71.0 \pm 19.55	–	41.3 \pm 3.26
Roots	57.2 \pm 8.05	13.9 \pm 1.63	12.7 \pm 1.47	–	19.1 \pm 6.90	–	12.2 \pm 1.42
<i>Viola</i>							
Shoot	–	–	108.7 \pm 8.65	67.4 \pm 3.92	–	–	10.0 \pm 1.14
Storage	32.5 \pm 2.84	–	11.0 \pm 1.21	14.0 \pm 1.45	31.0 \pm 4.10	–	19.8 \pm 1.76
Roots	109.0 \pm 7.00	–	22.5 \pm 2.03	22.7 \pm 2.00	35.4 \pm 4.6	–	32.3 \pm 2.81
<i>Tiarella</i>							
1997 shoot	–	32.4 \pm 7.71	131.1 \pm 15.60	–	92.8 \pm 13.50	64.4 \pm 11.25	41.8 \pm 15.44
1998 shoot	–	–	–	–	–	–	14.0 \pm 1.96
Rhizome	–	47.6 \pm 6.16	16.7 \pm 2.12	–	32.8 \pm 13.56	12.5 \pm 2.84	16.0 \pm 2.05
Roots	–	121.1 \pm 12.43	35.3 \pm 5.63	–	25.6 \pm 4.91	10.1 \pm 1.90	14.0 \pm 2.86

*Label in senesced leaves ($6.0 \pm 1.04 \mu\text{g g}^{-1}$); assumed to be lost from the plant.

Table 3 Apparent N resorption from leaves of *Allium* and *Tiarella*. The differences in N content between green and shed leaves were significant for both species using a Student's *t*-test ($P < 0.001$; $n = 5$). Values for leaf N are means \pm 1 SE

	Green leaf N (mg g ⁻¹)	Shed leaf N (mg g ⁻¹)	Resorption efficiency (%)
<i>Allium</i>	49.8 \pm 2.24	14.8 \pm 1.34	69.8
<i>Tiarella</i>	29.1 \pm 0.57	23.1 \pm 0.84	20.6

resources (Bloom *et al.* 1985). Although in *Tiarella* and *Allium* physiological processes measured over short time scales appear to violate this assumption, the whole plant over its entire annual growth cycle does not. The very low physiological activity of *Tiarella* leaves relative to its roots is compensated for by a greater allocation to leaves relative to roots. In contrast, *Allium* maintains its balance via differences in above- and below-ground phenology, and an ability to store and remobilize N (Tables 2 & 3).

While both high resorption efficiency and extended leaf lifespan are viewed as mechanisms of increasing MRT (Eckstein *et al.* 1999), differences here were determined primarily by differences in resorption efficiency, and MRT was actually inversely related to leaf lifespan. Despite having the shortest leaf lifespan, *Allium*'s efficient resorption and storage of leaf N resulted in very low annual N losses. In contrast, *Tiarella*'s poor resorption efficiency resulted in large losses of leaf N, even though it holds its leaves for the entire growing season. Although we are limited by a lack of data regarding leaf shedding in *Viola*, it appears from the changes in ¹⁵N pools (Table 2), and the autumnal increase in storage pools of N (Fig. 5), that *Viola* falls between *Allium* and *Tiarella* in its efficiency of leaf N resorption, contributing to its intermediate MRT. The unexpected inverse relationship between leaf lifespan and MRT may be explained by the fact none of these species has leaves that persist through more than one growing season, thus constraining any positive effects of leaf longevity on MRT. Furthermore, longer leaf display may actually increase the opportunity for N losses from the plant via leaching, particularly given the high leaf concentrations of soluble NO₃ in *Viola* and *Tiarella*.

Patterns of tissue phenology and N allocation clearly exert more influence over A than does leaf N concentration. High springtime A in *Allium* and *Tiarella* is associated with a higher proportional allocation of N to shoots, relative to *Viola* (Fig. 5), rather than higher leaf N concentration (similar in *Viola* and *Allium*, lower in *Tiarella*). Differences in springtime A should approximate differences in maximal, instantaneous A, given that this period has light, temperature and moisture conditions that are the most conducive to growth, but in this rapidly changing environment, annual and springtime N productivity followed very different patterns among species. Not surprisingly, extended,

slow C assimilation (*Tiarella*) resulted in greater annual N productivity than rapid C assimilation confined to a brief window of time (*Allium*).

Berendse & Aerts (1987) postulated that there were evolutionary trade-offs between traits leading to high A and those leading to high MRT, such that low-nutrient habitats should be dominated by plants with high MRT and low A whereas high-nutrient habitats should be dominated by plants with high A and low MRT. Even though our three species co-occur in a single high-nutrient habitat, their differences in life history still produced the expected negative relationship between A and MRT (Table 1). In fact, we found nearly as much variation in A and MRT among co-occurring species of differing phenologies as other researchers have found among species associated with habitats of widely varying N availability. Compare the 2.5-fold difference in A, and 1.7-fold difference in MRT, between *Tiarella* and *Allium* to the 2.3-fold difference in A, and a 1.9-fold difference in MRT, between a perennial grass species associated with acidic, nutrient-poor meadows and one associated with heavily fertilized pastures (Vázquez de Aldana & Berendse 1997).

Overall, the below-ground resource acquisition by deciduous-forest herbs does not relate to above-ground resource acquisition in nearly as clear-cut a manner as predicted, i.e. physiological rates of N uptake do not simply mirror rates of photosynthesis. However, patterns of allocation to, and phenology of, above- vs. below-ground structures appeared to compensate for disjunctions in physiology, resulting in relatively balanced C and N acquisition in all three species. Finally, life-history adaptations that appear to be related primarily to light availability also have important consequences for plant nitrogen economy.

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