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Rhizosphere control of soil nitrogen cycling: a key component of plant economic strategies

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Summary

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- Understanding how plant species influence soil nutrient cycling is a major theme in terrestrial ecosystem ecology. However, the prevailing paradigm has mostly focused on litter decomposition, while rhizosphere effects on soil organic matter (SOM) decomposition have attracted little attention.
- Using a dual ¹³C/¹⁵N labeling approach in a ‘common garden’ glasshouse experiment, we investigated how the economic strategies of 12 grassland plant species (graminoids, forbs and legumes) drive soil nitrogen (N) cycling via rhizosphere processes, and how this in turn affects plant N acquisition and growth.
- Acquisitive species with higher photosynthesis, carbon rhizodeposition and N uptake than conservative species induced a stronger acceleration of soil N cycling through rhizosphere priming of SOM decomposition. This allowed them to take up larger amounts of N and allocate it above ground to promote photosynthesis, thereby sustaining their faster growth. The N₂-fixation ability of legumes enhanced rhizosphere priming by promoting photosynthesis and rhizodeposition.
- Our study demonstrates that the economic strategies of plant species regulate a plant–soil carbon–nitrogen feedback operating through the rhizosphere. These findings provide novel mechanistic insights into how plant species with contrasting economic strategies sustain their nutrition and growth through regulating the cycling of nutrients by soil microbes in their rhizosphere.

Introduction

Across gradients of nutrient availability shaped by parent material, climate, pedogenesis and disturbance, plants have evolved sets of adaptive traits (Ordoñez *et al.*, 2009; Maire *et al.*, 2015). These trait syndromes form a spectrum of economic strategies along the tradeoff between acquisition and conservation of resources (Aerts & Chapin, 2000; Grime, 2001; Craine, 2009; Reich, 2014). Plant species of divergent economic strategies in turn reinforce existing patterns of nutrient availability by creating positive feedbacks to nutrient cycling (Hobbie, 1992). Plants can influence nutrient cycling both directly by their uptake, use and loss of nutrients, and indirectly by affecting soil decomposer activity and organic matter decomposition. Resource-acquisitive species adapted to nutrient-rich habitats are characterized by fast growth, high rates of photosynthesis and quick nutrient uptake, while resource-conservative species adapted to nutrient-poor habitats are characterized by slow growth and low rates of respiration and biomass turnover (Lambers & Poorter, 1992). The economic strategies of plant species also have ‘afterlife’ effects on the cycling of their own litter, with acquisitive species producing litter that decomposes quicker relative to conservative species (Freschet *et al.*, 2012).

So far, most studies of plant species effects on soil nutrient cycling have focused on litter decomposition (Berendse, 1994; Wardle *et al.*, 2004; Hobbie, 2015). However, soil organic matter (SOM) stabilized in mineral soil horizons represents a large pool of soil nutrients and contributes significantly to ecosystem nutrient supply (Jilling *et al.*, 2018). Nitrogen (N) is an important nutrient limiting plant growth in terrestrial ecosystems worldwide (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008). Because fresh litter is usually N-poor relative to their consumers, decomposer microbes retain rather than mineralize organic N from litter and immobilize mineral N from the surrounding soil during the early stages of litter decomposition (Parton *et al.*, 2007; Mooshammer *et al.*, 2014). Microbial N sequestration can therefore impair short-term positive feedbacks to soil N availability that operate through litter decomposition (Hodge *et al.*, 2000; Knops *et al.*, 2002; Craine, 2009). Conversely, SOM decomposition can promote N mineralization over immobilization because SOM stabilized in mineral soil horizons is usually N-rich (Mooshammer *et al.*, 2014). The prevailing paradigm based on litter feedbacks thus requires revision to better understand how plant species influence soil nutrient cycling and their own nutrient supply by affecting the decomposition not only of litter but also of SOM (Hobbie, 2015).

An important mechanism through which plants influence SOM decomposition is the allocation of photosynthate-carbon (C) to soil by their living roots via rhizodeposition (Farrar *et al.*, 2003; Jones *et al.*, 2004; Pausch & Kuzyakov, 2018). Rhizodeposits are known to commonly accelerate the decomposition of native SOM by stimulation of microbial exoenzyme production and disruption of mineral–organic associations (Cheng & Kuzyakov, 2005; Keiluweit *et al.*, 2015; Shahzad *et al.*, 2015). This phenomenon, known as the rhizosphere priming effect (Cheng *et al.*, 2014), is usually associated with enhanced gross rates of soil N mineralization, faster microbial biomass turnover and higher N availability for plant uptake (Dijkstra *et al.*, 2009; Zhu *et al.*, 2014; Yin *et al.*, 2018, 2019). Considering rhizosphere processes is thus essential for explaining soil N dynamics and plant N nutrition (Frank & Groffman, 2009; Finzi *et al.*, 2015; Moreau *et al.*, 2019).

The question of how plant economic strategies influence SOM decomposition via rhizosphere processes has attracted increasing interest (Bardgett *et al.*, 2014). It has been found that acquisitive species are associated with higher rates of rhizodeposition relative to conservative species (Kaštovská *et al.*, 2015; Guyonnet *et al.*, 2018; Henneron *et al.*, 2020). In a recent study, we have shown that this higher rhizodeposition by acquisitive species leads to faster soil C dynamics through rhizosphere priming of SOM decomposition (Henneron *et al.*, 2020). However, the effects of plant economic strategies on soil N cycling through rhizosphere priming remains largely unexplored, as most rhizosphere priming studies on soil N cycling to date have been limited to a small species pool without explicit consideration of plants traits (Dijkstra *et al.*, 2009; Zhu *et al.*, 2014; Yin *et al.*, 2018). Although the potential importance of rhizosphere processes for plant species effects on soil nutrient cycling has long been hypothesized (Hobbie, 1992), empirical evidence in support of this theory across multiple species is still lacking (Hobbie, 2015).

Using a ‘common garden’ glasshouse experiment, we studied the effects on soil N cycling of 12 grassland plant species (graminoids, forbs and legumes) selected to form a gradient of plant economic strategies. Plants were grown in a nutrient-rich grassland soil and labeled with a ^{13}C continuous-labeling method. We built up on a previous study in which plant and soil C cycling properties such as plant productivity, metabolic activity and photosynthesis, C rhizodeposition and native soil C mineralization were measured (Henneron *et al.*, 2020). Here, we quantified the effect of plant species on soil N cycling by measuring the gross rates of soil N mineralization and immobilization fluxes using a ^{15}N pulse-labeling of the soil, and by measuring the size and turnover of soil mineral and microbial N pools, and plant N uptake. We then explored the relationship of soil N cycling with plant economic traits, and how this relationship is coupled with plant N uptake as well as plant and soil C cycling properties. Together, this allowed us to investigate how the economic strategies of plant species drive soil N cycling via rhizosphere processes, and how this in turn affects plant N acquisition and growth. We tested two hypotheses (see Fig. 1): resource-acquisitive species with higher rates of photosynthesis, C rhizodeposition and N

uptake induce stronger acceleration of soil N cycling through rhizosphere priming of SOM decomposition relative to resource-conservative species; and this faster soil N cycling in turn allows acquisitive species to take up larger amounts of N and allocate this N above ground to promote C acquisition by photosynthesis, thereby sustaining their faster growth. Addressing these hypotheses in combination provides new insights into how plant species with contrasting economic strategies sustain their nutrition and growth by regulating the cycling of soil nutrients in their rhizosphere.

Materials and Methods

Experimental design and set-up

We established a ‘common garden’ glasshouse experiment including 12 common European grassland species: four C_3 grasses (*Anthoxanthum odoratum* L., *Festuca rubra* L., *Nardus stricta* L.,

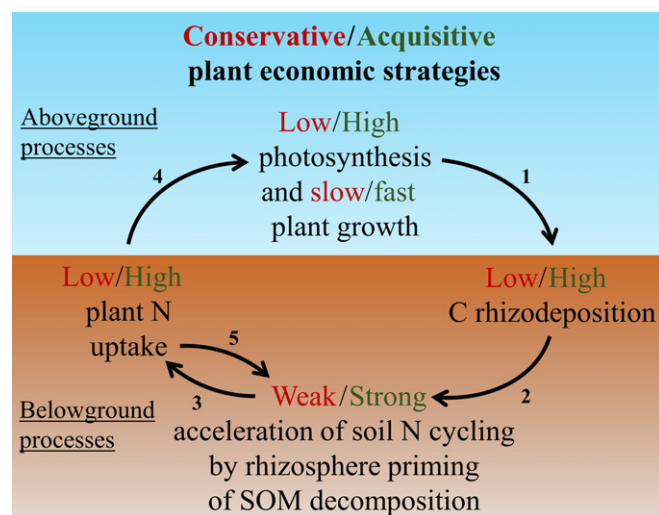


Fig. 1 Conceptual model showing how the economic strategies of plant species control soil nitrogen (N) cycling via rhizosphere processes, which in turn affects plant N acquisition and growth. The arrows represent the flow of causality. Ecosystem process rates are indicated in red (left) for the resource-conservative strategy and in green (right) for the resource-acquisitive strategy. (1) Acquisitive species are associated with higher carbon (C) fixation by photosynthesis relative to conservative species, which allows greater allocation of photosynthate-C to soil by rhizodeposition. Among the most important rhizodeposits are carbohydrates which provide energy for the production of exoenzymes catalyzing soil organic matter (SOM) decomposition by soil microbes, and organic acids which release SOM from protective associations with minerals. (2) Higher rhizodeposition of acquisitive species therefore causes stronger acceleration of soil N cycling through rhizosphere priming of SOM decomposition; this is related to faster gross N mineralization, and faster turnover of the mineral and microbial N pools. (3) Faster soil N cycling in their rhizosphere in turn allows acquisitive species to acquire larger amounts of N by root uptake. (4) This N is then allocated above ground to promote higher C acquisition by photosynthesis, thereby sustaining the faster growth of acquisitive species. (5) Higher plant N uptake by acquisitive species further stimulates rhizosphere priming by imposing more N-limiting growth conditions for soil microbes, thereby leading to greater microbial mining of N from SOM.

Poa trivialis L.), four nonleguminous forbs (*Chamerion angustifolium* (L.) Holub, *Plantago lanceolata* L., *Rumex acetosa* L., *Taraxacum officinale* (L.) Weber) and four legumes (*Lotus corniculatus* L., *Melilotus albus* Medik., *Trifolium repens* L., *Vicia cracca* L.). In each functional group, the species were selected based on *a priori* trait values to form a gradient of plant economic strategies (Henneron *et al.*, 2020).

The soil used is a nutrient-rich andosol, with a high SOM content and a low C : N ratio, collected from a seminatural grassland site in Laqueuille, Auvergne, France (45°38'N, 2°44'E, 1040 m elevation). We separately sampled and sieved (4 mm) the three top mineral soil layers (0–20, 20–40 and 40–60 cm). The main soil properties of the 0–20 cm layer are: soil C, 91.4 g kg⁻¹; soil C : N, 9.80; δ¹³C –26.70‰; pH, 5.26; texture, loam. Forty bottom-capped PVC pots (diameter 10 cm, height 60 cm), hereafter microcosms, were filled with fresh soil of each layer according to the initial stratification and bulk density of each layer. The microcosms were then weighed after abundant watering and 48 h of water percolation to measure the soil water-holding capacity (WHC). This also allowed leaching out of the mineral N that could have accumulated in the soil following its sampling and sieving. For each of the 12 species, three microcosms were sown to a density of seven and four plants per microcosm for grass and eudicot species, respectively. Four pots were kept unsown as unplanted control soil.

Immediately after *in situ* germination, the 40 microcosms were transferred in late August 2016 to a glasshouse exposed to natural light and temperature conditions (Clermont-Ferrand, temperate semicontinental climate). The experiment was performed for 256 d, until early June 2017. The glasshouse was coupled to a ¹³C continuous-labeling system (Henneron *et al.*, 2020). Briefly, ¹³C-depleted air was produced by injecting fossil fuel-derived CO₂ (δ¹³C = –35.23 ± 0.02‰) into CO₂-free air to reach ambient CO₂ concentration (400 ppm). The glasshouse was continuously supplied with ¹³C-depleted air during daytime. Soil water content was monitored daily with soil moisture sensors inserted to 5 cm depth, and drip irrigation was adjusted to maintain soil moisture around 85% of WHC. Senesced above-ground plant material lying on the soil surface was regularly collected to ensure that plants influenced soil properties exclusively by their roots.

Plant–soil microcosm CO₂ fluxes

For each microcosm, the plant–soil system respiration, corresponding to ecosystem dark respiration, was measured by incubation throughout spring on days 181, 209, 230 and 251 after planting. After ensuring similar soil moisture conditions to 85% WHC, each microcosm was then sealed in an opaque, airtight PVC chamber and incubated for 24 h in temperature-controlled conditions (21.5°C). At the end of incubation, the chamber gas was sampled and its CO₂ concentration and δ¹³C signature were measured using a gas chromatograph (Clarus 480, Perkin Elmer, Waltham, MA, USA) and an isotope laser spectrometer (CRDS Analyser, Picarro, Santa Clara, CA, USA). The continuous labeling of plants with ¹³C-depleted air allowed us to partition soil-

derived (*R*_{soil}) and plant-derived (*R*_{plant}) CO₂ sources into the ecosystem dark respiration using isotopic partitioning equations, as described by Henneron *et al.* (2020). *R*_{plant} represents the plant's metabolic activity, including both plant autotrophic respiration and soil microbial heterotrophic respiration derived from rhizodeposits. *R*_{soil} represents the soil microbial heterotrophic respiration derived from the mineralization of native soil C. We calculated the cumulative *R*_{soil} and *R*_{plant} by multiplying the average daily rate of CO₂ flux by the time interval between two sampling dates, and by adding the preceding CO₂ flux. The flux of native soil C mineralization derived from rhizosphere priming of SOM decomposition (soil C_{primed}) was calculated as the difference in *R*_{soil} between the planted microcosm and the average of unplanted controls (*R*_{soil-unplanted}). Supporting Information Methods S1 provides further methodological details on these measurements.

Plant and soil properties

At the end of the experiment, we separated harvested plants into above-ground materials, including leaves and stems, and below-ground materials, including rhizomes, tap roots and fine-roots. For each of the three soil layers (0–20, 20–40 and 40–60 cm depth), we separated soil and roots by passing the soil through a 2-mm sieve. Roots retained after sieving and all visible roots in sieved soil were carefully handpicked and washed. We carefully collected as much rhizosphere soil as possible by gently shaking off soil adhering to roots. Fresh soil was then immediately stored at 4°C until further analyses to minimize the mineralization of labile rhizodeposits. Soil analyses were limited to the top soil layer (0–20 cm) because most of living root effects on soil N and C cycling was probably concentrated in this layer (Finzi *et al.*, 2015).

Plant materials were oven-dried (48 h, 60°C), weighed, ground and analysed separately for %C, %N, δ¹³C and δ¹⁵N using an elemental analyzer coupled to an isotope-ratio mass spectrometer (IRMS, Elementar, Langensfeld, Hesse, Germany). The size of total, above-ground and below-ground plant N pools (N_{plant}, N_{shoot} and N_{root}) was calculated by multiplying their respective biomass and N concentration. For legumes, we separated plant N acquisition derived from soil N and from atmospheric-N₂ fixation in root nodules using the natural ¹⁵N abundance method (Unkovich *et al.*, 2008). The amount of plant N derived from soil N (N_{plant - soil}) was calculated using the following equation based on a two-source isotopic mixing model:

$$N_{\text{plant-soil}} = N_{\text{plant}} \times \left(1 - \frac{\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{reference}} - B} \right) \quad \text{Eqn 1}$$

where N_{plant} and δ¹⁵N_{legume} are the total amount and δ¹⁵N of shoot N of legume plants, δ¹⁵N_{reference} is the shoot δ¹⁵N of non-N₂-fixing 'reference' plants, and *B* is the isotope fractionation factor associated with N₂-fixation. δ¹⁵N_{reference} was the mean δ¹⁵N of the eight non-N₂-fixing species (3.25 ± 0.35‰ (SD)). The *B* value was –1.48‰ for *T. repens* based on the literature (Unkovich *et al.*, 2008). Because no *B* values were available for

the three remaining legume species, we calculated a mean B value ($-0.67 \pm 0.49\%$ (SD), range $[-1.48; -0.12]$) from 11 temperate grassland legume species with a B value available in the literature (Unkovich *et al.*, 2008). To assess the uncertainty in $N_{\text{plant-soil}}$ quantification for these three legume species, we performed a sensitivity analysis of 1‰ variation in B . The uncertainty in $N_{\text{plant-soil}}$ quantification remained small for *M. albus* (12.9%) and *V. cracca* (25.8%), but was high for *L. corniculatus* (96.4%, see Table S1). We therefore chose to discard *L. corniculatus* from the $N_{\text{plant-soil}}$ dataset. Plant N uptake rate was calculated by dividing $N_{\text{plant-soil}}$ by the number of days since planting.

We also measured additional plant C cycling properties, including above-ground, below-ground and fine-root net primary productivity (ANPP, BNPP and fine-root NPP) and canopy photosynthesis (A_{canopy}) at harvest, as described by Henneron *et al.* (2020). Methods S1 gives further methodological details on these measurements.

Gross fluxes of soil N mineralization and immobilization were quantified by the ^{15}N pool dilution method (Murphy *et al.*, 2003), which provides deeper insights into soil N cycling and N availability to plants than the classical method of assessing net N mineralization (Hart *et al.*, 1994; Schimel & Bennett, 2004; Frank & Groffman, 2009). Because our soil is a nutrient-rich andosol, with a high SOM content and low C : N ratio, we assume that amino acid uptake by plants was of minor importance (Schimel & Bennett, 2004; Kuzyakov & Xu, 2013). The day following harvest, fresh soil (100 g equivalent dry mass) was ^{15}N labeled by spreading it thinly, and spraying it with a $^{15}\text{NH}_4\text{Cl}$ solution (30 mg N- NH_4^+ kg $^{-1}$ soil enriched at 25 atom% ^{15}N) using an atomizer. The soil was then mixed, and two aliquots of 10 g were incubated at 21.5°C, 85% WHC. After 2 and 26 h, mineral N ($\text{NH}_4^+ + \text{NO}_3^-$) was extracted in 2 M KCl and its concentrations was measured using a continuous flow analyzer (AA3, Bran + Luebbe, Norderstedt, Germany). We collected NH_4^+ using a microdiffusion method consisting of trapping NH_4^+ over 7 d into H_2SO_4 -acidified filter paper disks after addition of MgO to an aliquot of the filtered extract inside airtight-sealed flasks. The $\delta^{15}\text{N}$ of NH_4^+ was measured using an elemental analyzer coupled to an IRMS. The gross rate of soil N mineralization ($N_{\text{mineralization}}$) was calculated using the equation of Kirkham & Bartholomew (1954):

$$N_{\text{mineralization}} = \frac{[\text{NH}_4^+]_0 - [\text{NH}_4^+]_t}{t} \times \frac{\log \frac{\delta^{15}\text{N}-\text{NH}_4^+_{t0}}{\delta^{15}\text{N}-\text{NH}_4^+_{t1}}}{\log \frac{[\text{NH}_4^+]_0}{[\text{NH}_4^+]_t}} \quad \text{Eqn 2}$$

where $[\text{NH}_4^+]_0$ and $[\text{NH}_4^+]_t$ are the concentrations of NH_4^+ at times 0 and t , $\delta^{15}\text{N}-\text{NH}_4^+_{t0}$ and $\delta^{15}\text{N}-\text{NH}_4^+_{t1}$ are the $\delta^{15}\text{N}$ of NH_4^+ at times 0 and t , and t is the incubation time. The measured flux of $N_{\text{mineralization}}$ is not modified by $^{15}\text{NH}_4$ supply (Murphy *et al.*, 2003), and the remineralization of labeled N was probably negligible during the 24 h incubation period (Braun *et al.*, 2018). Net rates of soil N mineralization were calculated from the changes in mineral N pool size over the course of the incubation period. The gross rate of N immobilization

($N_{\text{immobilization}}$) was calculated as the difference between gross and net N mineralization. Because $N_{\text{immobilization}}$ can be enhanced by the supply of NH_4^+ during the labeling, this flux was probably overestimated and considered only as potential flux hereafter.

The soil microbial biomass N ($N_{\text{microbial}}$) was measured by the chloroform-fumigation-extraction method (Brookes *et al.*, 1985). A 10 g aliquot of fresh soil was extracted in 0.5 M K_2SO_4 . A second set of samples was placed in a vacuum desiccator and fumigated with chloroform for 24 h before K_2SO_4 extraction. After oxidation of dissolved organic N by persulfate digestion, total dissolved N was measured using a continuous-flow analyzer as described above. $N_{\text{microbial}}$ was calculated from the differences between total dissolved N concentrations in the fumigated and the unfumigated samples using an extraction efficiency factor of 0.54 (Brookes *et al.*, 1985). Soil mineral N (N_{mineral} , $\text{NH}_4^+ + \text{NO}_3^-$) concentration was measured from 25 g of fresh soil after extraction in 2 M KCl as described above. Turnover rates of the N_{mineral} and $N_{\text{microbial}}$ pools ($\text{TR}-N_{\text{mineral}}$ and $\text{TR}-N_{\text{microbial}}$) were calculated by dividing gross $N_{\text{mineralization}}$ by each respective pool size (Hart *et al.*, 1994). Because this calculation assumes that gross $N_{\text{mineralization}}$ represents the only N efflux from microbial biomass while the microbial N efflux to SOM could be not negligible, $\text{TR}-N_{\text{microbial}}$ is probably underestimated.

We also measured additional soil C cycling properties, including soil heterotrophic respiration of new root-derived soil C ($R_{\text{H-C}_{\text{new}}}$) and native soil C ($R_{\text{H-C}_{\text{native}}}$), soil microbial biomass C ($C_{\text{microbial}}$), microbial metabolic quotient ($q\text{CO}_2$), and new root-derived soil C (C_{new}), as described by Henneron *et al.* (2020). Methods S1 provides further methodological details on these measurements. The C : N ratio of SOM mineralization ($C : N_{\text{mineralization}}$) was calculated as the ratio $R_{\text{H-C}_{\text{native}}} : \text{gross } N_{\text{mineralization}}$ (Murphy *et al.*, 2015; Yin *et al.*, 2018).

Plant economic traits

For each microcosm, we measured five plant economic traits related to key components of plant functioning (Henneron *et al.*, 2020): absolute growth rate (AGR), related to plant productivity; shoot : root ratio (S : R), related to plant biomass allocation; leaf light-saturated photosynthetic rate per mass (A_{leaf}), related to leaf photosynthetic activity; root dark respiration rate per mass (R_{root}), related to root metabolic activity; and root length density (RLD), related to soil exploration by roots. All traits were measured using standard methods described in Methods S1.

Statistical analyses

Because our study focuses on interspecific differences, species mean (the mean of the three microcosm replicates of a given species) was used as the statistical unit in all analyses ($n = 12$ species), unless otherwise specified. The normal distribution and homogeneity of variances of the model residuals were checked and data were log-transformed when necessary. All analyses were performed using R v.3.4.3 (R Core Team, 2017). Methods S1 gives further methodological details on these analyses.

We tested the effects of species identity (Sp, $n = 3$ microcosms per species) and functional group (FG, $n = 4$ species per functional group) on soil and plant N cycling properties using one-way ANOVAs with either Sp or FG as the fixed factor. Post-hoc comparisons of means were performed using Tukey's honest significant difference (HSD) tests.

To investigate the effect of plant economic strategies on soil N cycling, we performed an ordination of soil N cycling properties constrained by plant economic traits using a redundancy analysis. Soil C cycling, and plant N and C cycling properties were fitted in the ordination space as passive variables to assess how they are associated with the relationship of soil N cycling with plant economic traits. Each soil N cycling property was also related to plant economic traits by multimodel inference. Selection of multiple regression models was performed based on the Akaike's Information Criterion corrected for small sample size (AICc) to establish a confidence set of models with $\Delta\text{AICc} < 2$. Correlations of soil N cycling properties with plant economic traits, plant N cycling properties, and plant and soil C cycling properties were evaluated using Pearson's correlation coefficients.

Bivariate relationships between key plant and soil C and N cycling properties were tested by ordinary least squares regressions. We tested both linear ($Y = a + bX$) and power ($Y = aX^b$) functions, where Y is the response variable and X is the predictor. Additionally, we performed a sensitivity analysis to test if the bivariate relationships were robust at the within functional group level, and not merely driven by differences among functional groups. Bivariate regressions were then fitted by linear mixed-effect models with functional group as a random factor, which allow the intercept to vary among functional groups.

Results

The 12 grassland plant species were characterized by divergent trait syndromes spread across a plant economics spectrum corresponding to the first axis of the redundancy analysis (Fig. 2). Plant species with a resource-acquisitive strategy were characterized by fast growth (AGR), high allocation of biomass above ground (S : R), high leaf photosynthetic activity (A_{leaf}) and high root metabolic activity (R_{root}). Plant species with a resource-conservative strategy featured an opposite trait syndrome.

We found that these plant economic strategies were strongly related to soil N cycling properties ($F_{5,30} = 3.9$, $P < 0.001$), explaining 39.4% of the variation (Fig. 2). The first axis of the redundancy analysis (RDA1) explained a large portion of the variation (24.5%, $F_{1,30} = 12.1$, $P < 0.001$), whereas the second axis explained only 10.7% ($F_{1,30} = 5.3$, $P = 0.089$). We found significant effects of species identity ($F_{11,24} = 3.1$, $P = 0.009$) and functional group identity ($F_{2,9} = 4.4$, $P = 0.046$, legumes < grasses) on axis 1 scores. $N_{\text{mineralization}}$, $\text{TR-N}_{\text{microbial}}$, $N_{\text{immobilization}}$, $\text{C} : \text{N}_{\text{microbial}}$ and $\text{TR-N}_{\text{mineral}}$ were on the positive side of RDA1, which reflected the acquisitive strategy, while $N_{\text{microbial}}$ and $\text{C} : \text{N}_{\text{mineralization}}$ were on the negative side of RDA1, which reflected the conservation strategy (Fig. 2; Table S2). We also found strong coupling with plant N cycling properties: N_{shoot} and N_{root} were related to the acquisitive strategy; soil C cycling

properties: R_{soil} and $R_{\text{H-C}_{\text{new}}}$ were related to the acquisitive strategy, while $\text{C}_{\text{microbial}}$ was related to the conservation strategy; and plant C cycling properties: R_{plant} , A_{canopy} and ANPP were related to the acquisitive strategy (Fig. 2; Table S2).

Absolute growth rate (AGR) was among the most important driver of all soil N cycling processes, except $\text{C} : \text{N}_{\text{mineralization}}$ (Tables S3, S4). However, other economic traits such as shoot : root ratio (S : R), leaf photosynthetic rate (A_{leaf}) and root respiration rate (R_{root}) were also important drivers of most soil N cycling processes, even after accounting for the AGR effect. These traits were stronger drivers than AGR for $N_{\text{mineralization}}$, $\text{C} : \text{N}_{\text{mineralization}}$, $\text{TR-N}_{\text{microbial}}$ and $\text{TR-N}_{\text{mineral}}$. For instance, $N_{\text{mineralization}}$ was strongly positively related to S : R and R_{root} after accounting for the moderate positive effect of AGR. Similarly, $\text{TR-N}_{\text{microbial}}$ was positively related to S : R and A_{leaf} after accounting for the positive effect of AGR. RLD never emerged as an important driver of soil N cycling properties.

Plant metabolic activity at the end of the experiment (final R_{plant}) was tightly related to plant economic traits (Fig. 2, multiple regression model for final R_{plant} : AGR, $\beta_{\text{st}} = 0.61$, % of $r^2 = 54$, $P < 0.001$; S : R, $\beta_{\text{st}} = 0.56$, $P < 0.001$, % of $r^2 = 37$; A_{leaf} , $\beta_{\text{st}} = 0.21$, $P = 0.001$, % of $r^2 = 3$; R_{root} , $\beta_{\text{st}} = -0.19$, $P < 0.001$, % of $r^2 = 5$; model $r^2 = 0.99$). R_{plant} was therefore used hereafter as a proxy for the plant's position along the plant economics spectrum, with plant species featuring high metabolic activity being associated with a resource-acquisitive strategy and plant species featuring low metabolic activity being associated with a resource-conservative strategy.

The gross rate of soil N mineralization was positively related to plant metabolic activity, showing a stronger acceleration of soil N mineralization by acquisitive species relative to conservative species (Fig. 3a). Gross N mineralization was also positively related to the rates of canopy photosynthesis (Fig. 3b), new root-derived soil C mineralization (Fig. 3c) and native soil C mineralization (Fig. 3d). Gross N mineralization was higher for legume species (Table S5), in relation to their higher rates of plant metabolic activity, canopy photosynthesis, new root-derived soil C mineralization and native soil C mineralization at the end of the experiment (Fig. 3).

All species greatly reduced the size of the mineral N pool and accelerated its turnover, but these effects were stronger for acquisitive than for conservative species (Fig. 4a,b). We also found higher potential gross rates of soil N immobilization for acquisitive species (Fig. S1a). Conservative species supported a larger microbial N pool size relative to the unplanted control, while the size of this pool remained little affected by acquisitive species (Fig. 4c). Acquisitive species were characterized by faster turnover of the microbial N pool ($\text{TR-N}_{\text{microbial}}$, Fig. 4d), and higher $\text{C} : \text{N}$ ratio of microbial biomass (Fig. S1b). Conversely, acquisitive species featured lower $\text{C} : \text{N}$ ratio of SOM mineralization ($\text{C} : \text{N}_{\text{mineralization}}$, Fig. S1c). Legume species had higher $\text{TR-N}_{\text{microbial}}$ and lower $\text{C} : \text{N}_{\text{mineralization}}$ (Table S5).

We also found that rhizosphere effects on soil N cycling had important consequences for plant N acquisition and growth. Plant N uptake was higher for acquisitive species, and was positively related to spring plant metabolic activity (Fig. 5a) and fine-

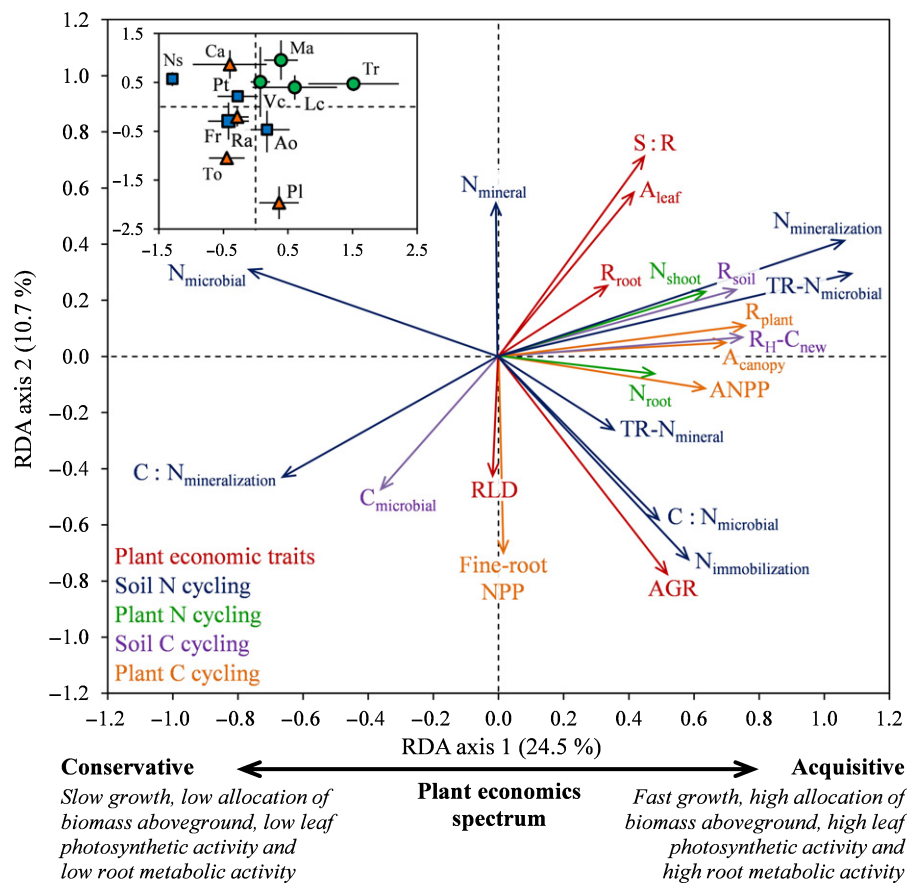


Fig. 2 Redundancy analysis of soil N cycling properties (blue arrows) constrained by plant economic traits (red arrows). Plant N cycling properties (green arrows), soil C cycling properties (purple arrows) and plant C cycling properties (orange arrows) were fitted in the ordination as passive variables. The coordinate means of each species are plotted in the inset and error bars represent \pm SE ($n = 3$). Functional groups (FG) are represented by blue squares, orange triangles and green circles for grass, forb and legume species, respectively. Plant economic traits as well as plant and soil C cycling properties are from Henneron *et al.* (2020). For simplicity, only the most relevant variables are shown here. For the coordinates of all variables, see Supporting Information Table S2. Plant and soil N cycling properties across species are shown in Tables S5 and S7. Plant economic traits: AGR, absolute growth rate; S : R, shoot : root ratio; A_{leaf} , leaf light-saturated photosynthetic rate per mass; R_{root} , root dark respiration rate per mass; RLD, root length density. Soil N cycling properties: $N_{\text{mineralization}}$, gross rate of soil N mineralization; C : $N_{\text{mineralization}}$, C : N ratio of native soil organic matter mineralization; $N_{\text{immobilization}}$, potential rate of mineral N immobilization; $N_{\text{microbial}}$, soil microbial biomass N; TR- $N_{\text{microbial}}$, turnover rate of soil microbial biomass N; C : $N_{\text{microbial}}$, C : N ratio of soil microbial biomass; N_{mineral} , soil mineral N; TR- N_{mineral} , turnover rate of soil mineral N. Plant N cycling properties: N_{shoot} and N_{root} , plant N acquisition allocated above ground and below ground, respectively. Soil C cycling properties: R_{soil} , respiration derived from native soil C mineralization at the end of experiment; $R_{\text{H-C}_{\text{new}}}$, heterotrophic respiration of new root-derived soil C; $C_{\text{microbial}}$, soil microbial biomass C. Plant C cycling properties: R_{plant} , respiration derived from C recently fixed by plants at the end of the experiment; A_{canopy} , canopy photosynthesis; ANPP and Fine-root NPP, above-ground and fine-root net primary productivity, respectively. Plant species: Ao, *Anthoxanthum odoratum*; Ca, *Chamerion angustifolium*; Fr, *Festuca rubra*; Lc, *Lotus corniculatus*; Ma, *Melilotus albus*; Ns, *Nardus stricta*; Pl, *Plantago lanceolata*; Pt, *Poa trivialis*; Ra, *Rumex acetosa*; To, *Taraxacum officinale*; Tr, *Trifolium repens*; Vc, *Vicia cracca*.

root production (Fig. S2a), but also to native soil C mineralization through rhizosphere priming during the early growing season (spring soil C_{primed} , Fig. 5b). A multiple regression model still showed a strong relationship of plant N uptake with spring soil C_{primed} after accounting for fine-root production (soil C_{primed} : $\beta_{\text{st}} = 0.69$, $P = 0.039$, % of $r^2 = 52$; fine-root production: $\beta_{\text{st}} = 0.52$, $P = 0.045$, % of $r^2 = 48$, model $r^2 = 0.67$). Furthermore, total plant N acquisition (derived from both N_2 -fixation and N uptake) was also positively related to spring soil C_{primed} , and this relationship remained strongly significant even after accounting for plant N_2 -fixing ability (Fig. S2b).

Importantly, plant N uptake allocated above ground was also positively related to soil C_{primed} (Fig. 5c). In turn, ANPP was

strongly positively related to plant N uptake allocated above ground (Fig. 5d), although it was only moderately positively related to plant N uptake ($\beta_{\text{st}} = 0.50$, $P = 0.073$, $r^2 = 0.31$).

The sensitivity analysis showed that the vast majority (16 out of 18) of significant bivariate regressions were robust within functional groups (Table S6), indicating that most relationships found were not confounded by the effect of any particular functional group, such as legume species with N_2 -fixing ability.

Discussion

To date, litter decomposition has been the primary focus of most studies investigating how plant species influence soil nutrient

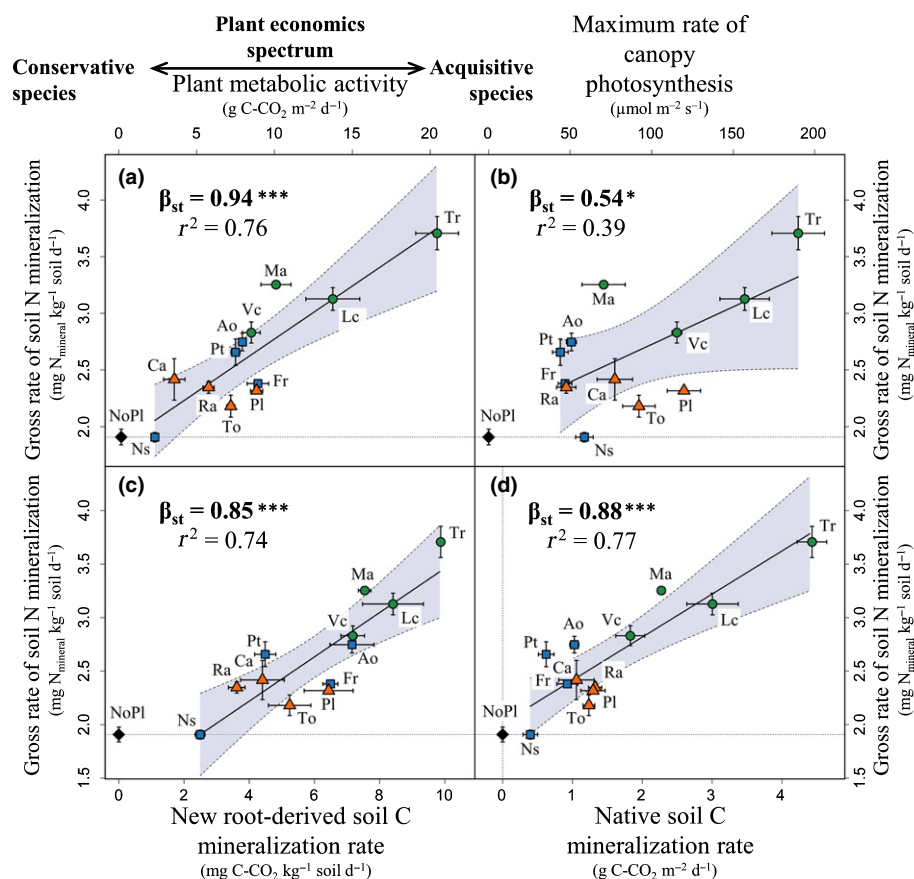


Fig. 3 Effects of plant economic strategies on soil N mineralization via rhizosphere priming. Relationships of the gross rate of soil N mineralization with (a) plant metabolic activity, corresponding to the respiration of C recently fixed by plants and used as a proxy of the plant economics spectrum; (b) plant C fixation by canopy photosynthesis; (c) new root-derived soil C mineralization, corresponding to the microbial utilization of rhizodeposit-C; and (d) native soil C mineralization. All these fluxes have been measured at the end of the experiment. The amounts of soil C and N primed represent the extra C and N mineralization relative to the unplanted control. The values for C cycling properties are from Hennen *et al.* (2020). The means of each treatment are plotted ($n = 3$ for each species; $n = 4$ for the unplanted control, NoPl), and error bars represent \pm SE. Functional groups are represented by blue squares, orange triangles and green circles for grass, forb and legume species, respectively. Regressions were performed using species means as the statistical unit ($n = 12$). The filled areas indicate 95% confidence intervals. β_{st} is the range-standardized regression coefficient (effect size). Plant species: Ao, *Anthoxanthum odoratum*; Ca, *Chamerion angustifolium*; Fr, *Festuca rubra*; Lc, *Lotus corniculatus*; Ma, *Melilotus albus*; Ns, *Nardus stricta*; Pl, *Plantago lanceolata*; Pt, *Poa trivialis*; Ra, *Rumex acetosa*; To, *Taraxacum officinale*; Tr, *Trifolium repens*; Vc, *Vicia cracca*. ***, $P < 0.001$, *, $P < 0.05$.

cycling (Hobbie, 2015). Here, we provide experimental evidence that rhizosphere processes are also major drivers of plant species effects on soil N cycling. Specifically, we show that the economic strategies of plant species shape soil N cycling by regulating rhizosphere priming of SOM decomposition, probably through controlling the allocation of photosynthate-C to soil via rhizodeposition and the uptake of N by roots. Consistent with our first hypothesis, we demonstrate that acquisitive plant species, which are characterized by higher rates of photosynthesis, C rhizodeposition and N uptake than conservative species, induce stronger acceleration of soil N cycling than conservative species (Figs 1, 6). This is linked to a higher gross rate of soil N mineralization, faster turnover of the mineral and microbial N pools, and reduced N sequestration in soil microbial biomass (Figs 2–4). This means that acquisitive species are associated with faster soil N cycling in their rhizosphere, as well as a tighter coupling of C and N cycling involving a trading of labile C to microbes against N to plants (Kuzakov & Xu, 2013). Importantly, the ability of

acquisitive species to speed up soil N cycling through rhizosphere priming was primarily related to their fast growth, which allows them to quickly reach high biomass after disturbance. However, it was also related to their high allocation of biomass above ground and high leaf photosynthetic activity leading to high photosynthesis, as well as their high root metabolic activity allowing high rhizodeposit-C supply and N uptake (Fig. 2; see also Hennen *et al.*, 2020).

The gross rate of soil N mineralization was controlled by plant metabolic activity, with acquisitive species inducing stronger stimulation of soil N mineralization than conservative species (Fig. 3a). Rhizosphere effects on soil N mineralization were driven by the supply and microbial utilization of rhizodeposit-C (Fig. 3b,c), and this root-induced soil N mineralization was mostly derived from SOM decomposition, rather than from fresh root litter or rhizodeposit decomposition (Fig. 3d). In support of the ‘microbial activation’ hypothesis (Cheng & Kuzyakov, 2005), these results showed that the rhizosphere priming of SOM

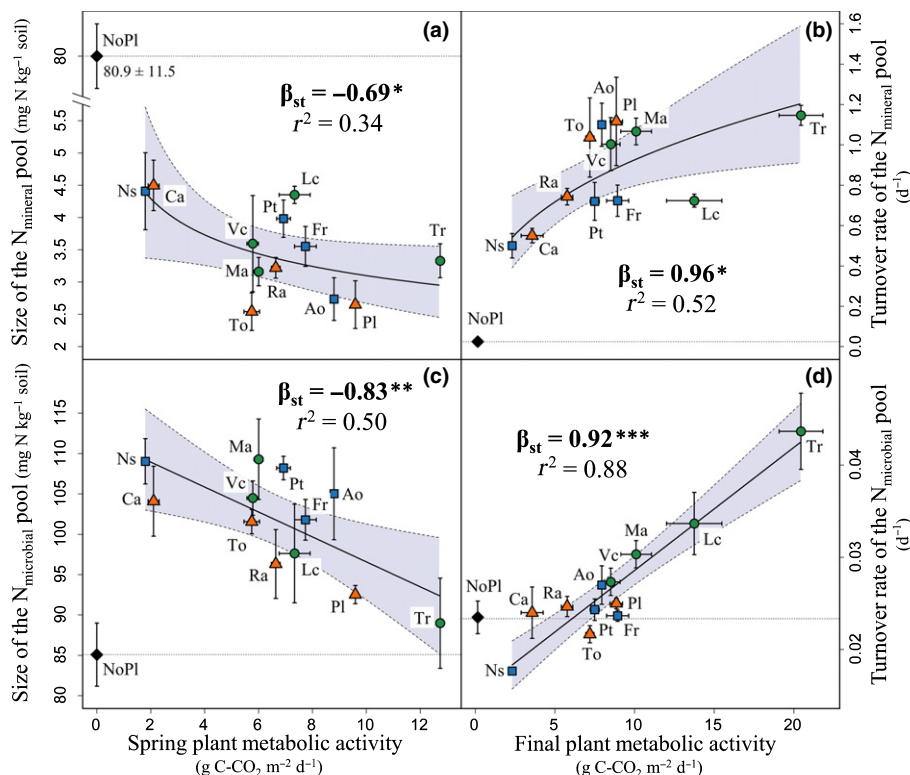


Fig. 4 Effects of plant economic strategies on the size and turnover of soil mineral and microbial N pools. Relationships of the mineral N (N_{mineral}) pool size (a) and turnover rate (b), and the microbial N ($N_{\text{microbial}}$) pool size (c) and turnover rate (d), with plant metabolic activity (R_{plant}), corresponding to the respiration of C recently fixed by plants and used as a proxy of the plant economics spectrum. Spring and final R_{plant} indicate the cumulative flux during the early growing season and the flux at the end of the experiment, respectively. Spring and final plant metabolic activities were related to the size and flux of N pools, respectively. The values for spring and final R_{plant} are from Henneron *et al.* (2020). The means of each treatment are plotted ($n = 3$ for each species; $n = 4$ for the unplanted control, NoPl), and error bars represent \pm SE. Functional groups are represented by blue squares, orange triangles and green circles for grass, forb and legume species, respectively. Regressions were performed using species means as the statistical unit ($n = 10$). The filled areas indicate 95% confidence intervals. β_{st} is the range-standardized regression coefficient (effect size). Plant species: Ao, *Anthoxanthum odoratum*; Ca, *Chamerion angustifolium*; Fr, *Festuca rubra*; Lc, *Lotus corniculatus*; Ma, *Melilotus albus*; Ns, *Nardus stricta*; Pl, *Plantago lanceolata*; Pt, *Poa trivialis*; Ra, *Rumex acetosa*; To, *Taraxacum officinale*; Tr, *Trifolium repens*; Vc, *Vicia cracca*. ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$.

decomposition is driven by the rhizodeposition of labile C compounds, such as carbohydrates providing energy for the microbial production of exoenzymes catalyzing SOM decomposition. The rhizodeposition of organic acids could further accelerate the decomposition of SOM by releasing it from protective associations with minerals (Keiluweit *et al.*, 2015).

The higher C rhizodeposition and rhizosphere priming by acquisitive species was also associated with greater depletion of the soil mineral N pool, probably partly due to higher plant N uptake (Figs 2, 4a, 5a). This supports the ‘microbial N mining’ hypothesis, which postulates that under N-limiting growth conditions, decomposer microbes use labile-C to produce exoenzymes catalyzing the decomposition of SOM to access the N it contains (Fontaine & Barot, 2005; Craine *et al.*, 2007). Accordingly, we found acquisitive species to cause a reduction of soil microbial biomass N and C as well as a faster turnover of the microbial biomass N (Figs 2, 4c,d), despite higher rhizodeposition of labile-C available for microbial growth. These results suggest that the roots of acquisitive species lead microbes to invest more of their resources into the production of exoenzymes at the expense of their growth (Schimel & Weintraub, 2003; Shahzad

et al., 2015). Furthermore, the lower C : N ratio of SOM mineralization found here for the acquisitive species suggests that they can promote the mobilization of SOM pools that are more N-rich (Murphy *et al.*, 2015). This could be potentially linked to the release of N-rich SOM from the disruption of mineral–organic associations by organic acid exudates (Keiluweit *et al.*, 2015; Jilling *et al.*, 2018). The higher C : N ratio of microbial biomass associated with acquisitive species also suggests that they promote fungi (Pausch *et al.*, 2015), whose biomass typically has a high C : N ratio (Strickland & Rousk, 2010), at the expense of bacteria. Interestingly, fungi have been associated with greater soil exploration and enzymatic ability for N mining of SOM than bacteria (Carney *et al.*, 2007; Fontaine *et al.*, 2011; Shahzad *et al.*, 2012).

Acquisitive species were also characterized by potentially higher gross rates of N immobilization, indicating that higher stimulation of microbial growth via rhizodeposition of labile C-rich compounds increases the immobilization of soil mineral N by microbes (Kuzakov & Xu, 2013). This raises the question of whether the potential enhancement of N availability for plants arising from rhizosphere priming could be impeded by greater

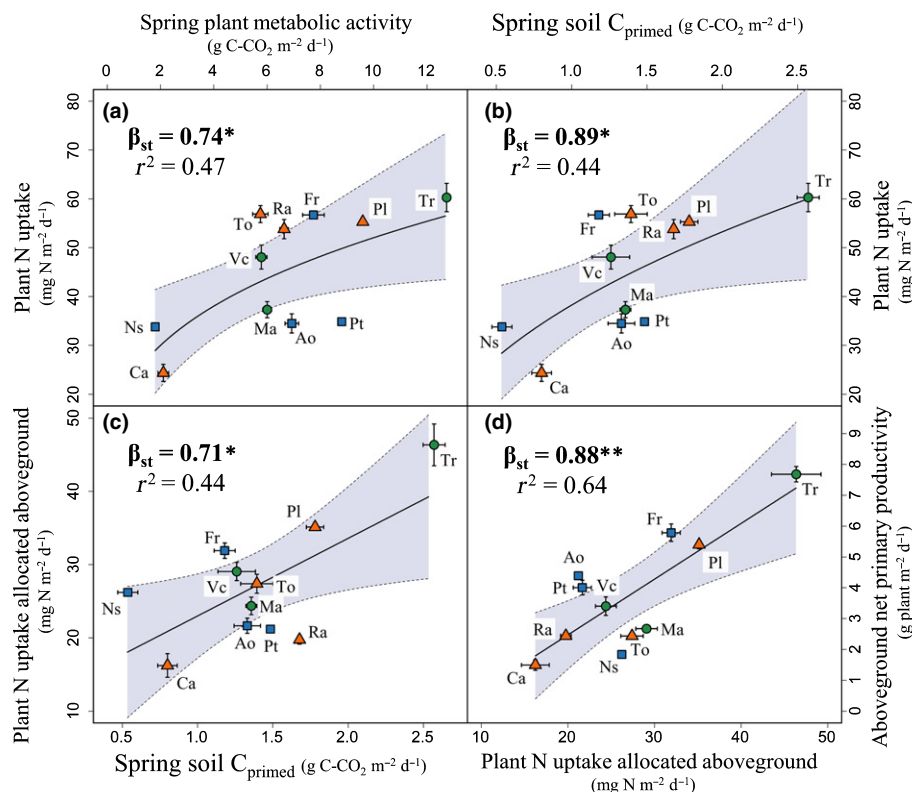


Fig. 5 Effects of plant economic strategies on plant nutrition and productivity via rhizosphere priming. Relationships of plant N uptake with (a) spring plant metabolic activity, corresponding to the respiration of C recently fixed by plants and used as a proxy of the plant economics spectrum; and (b) spring soil C_{primed} , corresponding to the flux of native soil C mineralization through rhizosphere priming of SOM decomposition. Relationships of plant N uptake allocated to above-ground biomass with (c) spring soil C_{primed} ; and (d) above-ground net primary productivity (ANPP). Spring soil C_{primed} was assumed to better represent the cumulative flux of soil N mineralization through rhizosphere priming during the early growing season than was the gross N mineralization flux measured at the end of the experiment (Dijkstra *et al.*, 2009; Henneron *et al.*, 2020). For legume species, we assessed plant N uptake by partitioning N acquisition into that derived from the root uptake of soil N vs from the fixation of atmospheric- N_2 using the natural ^{15}N abundance method (Unkovich *et al.*, 2008). The values for spring R_{plant} , spring soil C_{primed} and ANPP are from Henneron *et al.* (2020). The means of each species are plotted ($n = 3$), and error bars represent \pm SE. Functional groups are represented by blue squares, orange triangles and green circles for grass, forb and legume species, respectively. Regressions were performed using species means as the statistical unit ($n = 11$). The filled areas indicate 95% confidence intervals. β_{st} is the range-standardized regression coefficient (effect size). Plant species: Ao, *Anthoxanthum odoratum*; Ca, *Chamerion angustifolium*; Fr, *Festuca rubra*; Ma, *Melilotus albus*; Ns, *Nardus stricta*; Pl, *Plantago lanceolata*; Pt, *Poa trivialis*; Ra, *Rumex acetosa*; To, *Taraxacum officinale*; Tr, *Trifolium repens*; Vc, *Vicia cracca*. **, $P < 0.01$, *, $P < 0.05$.

competition with microbes (Knops *et al.*, 2002; Dijkstra *et al.*, 2013). However, acquisitive species were associated with a smaller size and faster turnover of the microbial N pool relative to conservative species (Fig. 4c,d).

This reduced N sequestration in microbial biomass for acquisitive species could be explained by several fundamental properties of the rhizosphere (Schimel & Bennett, 2004; Cheng & Gershenson, 2007; Frank & Groffman, 2009). First and most importantly, stronger stimulation of microbial growth by higher rhizodeposition could in turn increase the grazing pressure by microbivore soil fauna such as protists, nematodes and microarthropods, thus releasing more microbial N into mineral forms according to the ‘microbial loop’ hypothesis (Moore *et al.*, 2003; Trap *et al.*, 2015). Second, microbial growth could be more N-limited due to higher supply of N-poor exudates and higher N uptake by roots (Cheng & Kuzyakov, 2005; Cheng & Gershenson, 2007). This is consistent with the higher C : N ratio of microbial biomass found here for acquisitive species. Third,

higher water uptake by roots could increase the frequency of soil drying–rewetting cycles, enhancing microbial mortality by hydric stress and higher exposure to faunal grazing by soil aggregate destruction (Cheng & Kuzyakov, 2005; Lu *et al.*, 2019). Given the much longer lifespan of roots relative to microbes and the net flow of nutrients from soil to roots, faster microbial turnover provides enhanced long-term opportunities for roots of acquisitive species to successfully compete for N against microbes (Schimel & Bennett, 2004; Schmidt *et al.*, 2007; Kuzyakov & Xu, 2013).

Interestingly, we found that conservative species supported a larger microbial biomass N and a much smaller mineral N pool relative to the unplanted soil, despite their slow growth and low N uptake (Fig. 4a,c). This provides evidence that low but consistent C rhizodeposition together with low plant N uptake prevents the decline in microbial biomass and associated accumulation of soil mineral N that is typically observed in long-term soil incubation in the absence of C supply as a result of energy limitation of microbial growth (Hart *et al.*, 1994). This microbial N retention

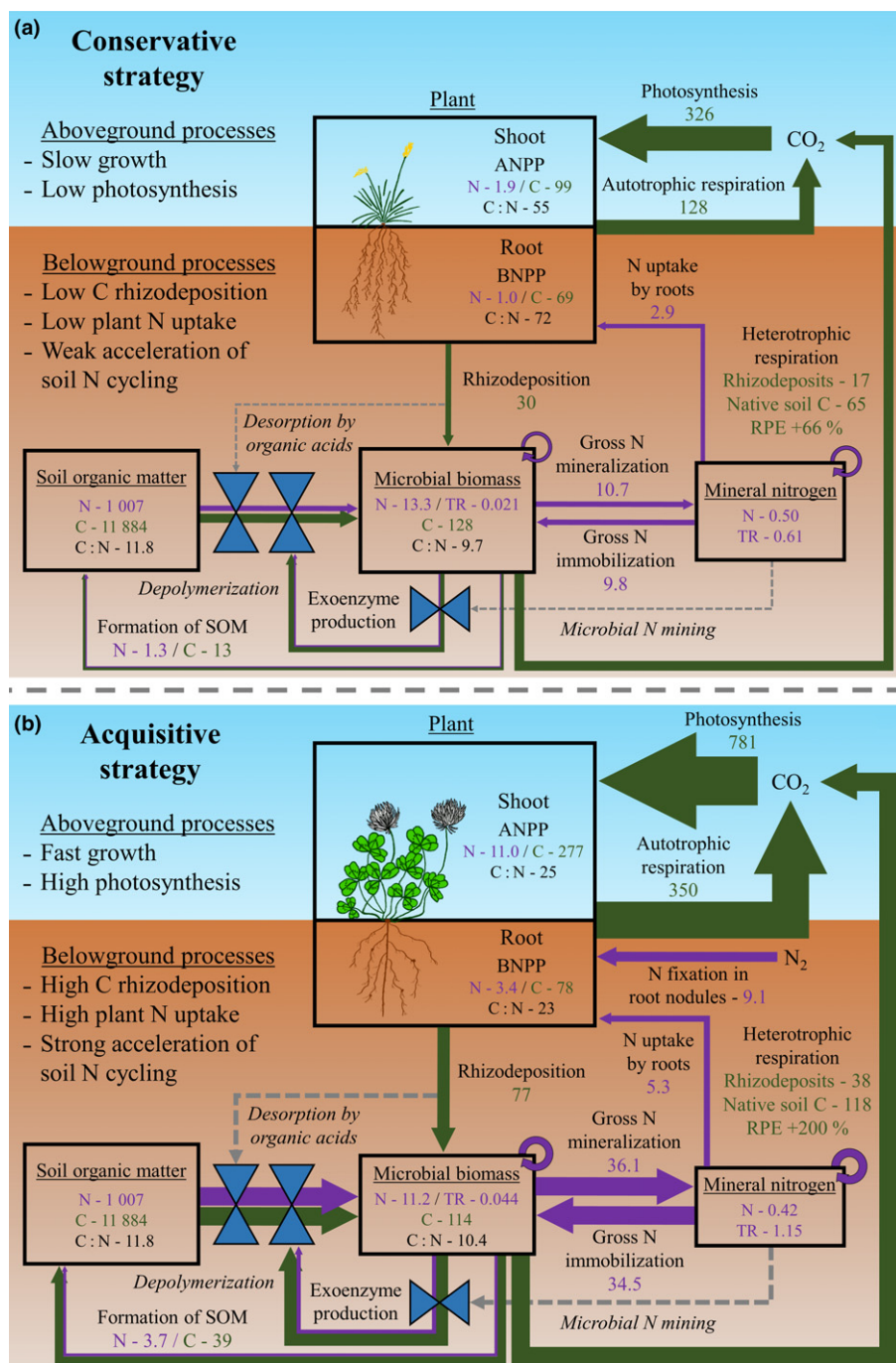


Fig. 6 Ecosystem nitrogen (N, in purple) and carbon (C, in green) budgets for conservative (a) and acquisitive (b) plant economic strategies. Boxes reflect pools (in g C or N m⁻²), while solid arrows reflect fluxes (in g C or N m⁻² month⁻¹). Circular arrows reflect pool turnover rates (TR, d⁻¹). Box sizes and arrow widths indicate quantitative differences in pool sizes and flux rates, respectively. Gray dashed or solid arrows connected to 'valves' (attached blue triangles) indicate regulation points. Pool sizes and flux rates of each strategy were derived from the values of representative species (*Trifolium repens* values for the acquisitive strategy and average values of *Nardus stricta* and *Festuca rubra* for the conservative strategy). The rhizosphere priming effect (RPE) is the change in native soil C mineralization rate relative to the unplanted control. ANPP, above-ground net primary productivity; BNPP, below-ground net primary productivity. For details about species selection and C and N budget computation, see Supporting Information Methods S1. Plant images by Alice Trotel.

mechanism could contribute to the ability of conservative species to protect ecosystems from N losses through leaching or denitrification (de Vries *et al.*, 2012). Overall, our results suggest that microbial biomass acts as both a sink and a source of available nutrients controlled by rhizosphere processes.

The effects of plant economic strategies on soil N cycling via rhizosphere processes had important consequences for plant nutrition and productivity. In support of our second hypothesis, the higher rhizosphere priming of SOM decomposition that we observed for acquisitive species had positive effects on the amounts of plant N taken up by roots and allocated above

ground (Fig. 5b,c), where it can in turn be used to support C acquisition by leaf photosynthesis (Wright *et al.*, 2004; Ollinger *et al.*, 2008). Higher rhizosphere priming of SOM decomposition by acquisitive species has been linked to their higher above-ground productivity (Henneron *et al.*, 2020) (see Fig. 2). Acquisitive species are commonly associated with lower allocation of biomass to fine-roots that intercept soil nutrients relative to conservative species (Hobbie, 1992; Lambers & Poorter, 1992), which could potentially impede their nutrition. However, we show here that higher rates of photosynthesis and photosynthate-C allocation to soil at the expense of fine-root growth allows

acquisitive species to enhance soil N cycling in their rhizosphere (Figs 1, 6). Because acquisitive species typically also have higher root N uptake capacity (Maire *et al.*, 2009), this faster soil N cycling increasing the supply of N available to plants in turn creates a positive feedback which sustains their higher above-ground productivity by allowing them to take up larger amounts of N and allocate it above ground to promote canopy photosynthesis (Figs 1, 5c,d) (Drake *et al.*, 2011; Phillips *et al.*, 2011).

Legumes were the functional group that induced the highest rhizosphere priming, although there was also substantial variation among legume species related to their contrasting economic strategies (Fig. 3). Nodulation has indeed been shown to enhance rhizosphere priming (Zhu & Cheng, 2012), probably because N₂-fixation reinforces their photosynthetic capacity and C rhizodeposition (Henneron *et al.*, 2020). It could appear paradoxical that legumes would mine SOM for N through rhizosphere priming despite their ability to rely on symbiotic associations with N₂-fixing bacteria for N acquisition. However, the energetic cost of fixing N₂ can be high relative to the cost of mineral N uptake (Vitousek & Howarth, 1991). As such, it is possible that once they have accumulated enough N in their canopy to allow high levels of photosynthesis and C rhizodeposition, legumes can then shift their N-acquisition strategy from N₂-fixation to the potentially less costly mineral N uptake pathway that is coupled to rhizosphere priming. Legumes may also rely on rhizosphere priming for the acquisition of other nutrients such as phosphorus, which are potentially limiting for plant growth and N₂-fixation (Vitousek & Howarth, 1991; van Groenigen *et al.*, 2006).

Several key questions remain to be addressed. For instance, we still need to test how rhizosphere effects on soil nutrient cycling interact with soil nutrient richness as shaped by plant economic strategies (Hobbie, 1992; Hobbie, 2015). High nutrient availability generally favors acquisitive species and their high-quality and nutrient-rich litter could contribute to the formation of N-rich SOM (Ordoñez *et al.*, 2009; Mueller *et al.*, 2015; Craig *et al.*, 2018), which can then be mined for N through rhizosphere priming for supply to plants, as observed here with a nutrient-rich soil. By contrast, low nutrient availability generally favors conservative species, and their low-quality and nutrient-poor litter could lead to the formation of N-poor SOM (Ordoñez *et al.*, 2009). Furthermore, some conservative species produce large amounts of secondary compounds such as tannins, which can protect organic N from decomposition by forming recalcitrant protein–tannin complexes (Northup *et al.*, 1998; Adamczyk *et al.*, 2019). The stimulation of soil N mineralization by rhizosphere priming of SOM decomposition that we observed in our nutrient-rich soil might therefore not efficiently operate in a nutrient-poor soil because decomposer microbes could retain rather than mineralize most primed organic N (Schimel & Bennett, 2004; Mooshammer *et al.*, 2014). High rhizodeposition by plants could instead promote enhanced N sequestration in microbial biomass, thereby further increasing N-limitation for plants (Díaz *et al.*, 1993), but empirical evidence for this is scarce. However, conservative species associated with ericoid mycorrhizal and ectomycorrhizal fungi could enhance their N acquisition in nutrient-poor soil by allocating photosynthate-C in exchange for N to

their mycorrhizal partners that have the enzymatic ability to mine organic N in nutrient-poor soils (Phillips *et al.*, 2011; Adamczyk *et al.*, 2019).

Our findings have important implications for our understanding of vegetation control over N cycling in terrestrial ecosystems. First, the strong linkage between plant physiological functioning and soil N biogeochemistry in the rhizosphere involves a much tighter spatiotemporal coupling of plant–microbe–soil interactions than do the ‘afterlife’ effects of litter decomposition (Bardgett *et al.*, 2005; Högborg & Read, 2006). This is essential for the ability of plants to control soil N cycling and supply in a way sustaining their nutrition (Aerts & Chapin, 2000). Second, our study provides empirical evidence that rhizosphere priming improves plant growth by providing roots with available N according to plant demand (Kuzyakov & Xu, 2013). This suggests that rhizosphere priming is adaptive and could have evolved as a mutualistic interaction in which the C cost of rhizodeposition for plants is balanced by benefits provided by root-associated decomposer microbes in terms of plant N nutrition (Lambers *et al.*, 2009; Cheng *et al.*, 2014). Overall, our findings suggest that rhizosphere processes enhance plant fitness, and could be involved in the evolutionary processes that shape the economic strategies of plant species related to their nutritional ecology (van Breemen & Finzi, 1998; Aerts & Chapin, 2000; Reich *et al.*, 2003). According to the ‘bank mechanism’ theory (Fontaine *et al.*, 2011), the high investment into C rhizodeposition coupled to high plant N uptake by acquisitive species gives them the ability to mobilize enough N from SOM through rhizosphere priming to sustain their fast N uptake and growth in nutrient-rich habitats. By contrast, the more parsimonious investment into C rhizodeposition coupled to low plant N uptake by conservative species could contribute, together with their production of low-quality and nutrient-poor litter, to enhancing soil N retention in microbial biomass and SOM in the long term (de Vries *et al.*, 2012). This could in turn prevent ecosystem N losses through leaching or denitrification and thereby benefit conservative species in nutrient-poor habitats (Northup *et al.*, 1998; Kuzyakov & Xu, 2013). Rhizosphere control of soil N cycling therefore represents a plant’s ‘extended phenotype’ (van Breemen & Finzi, 1998), and its regulation could be a powerful mechanism through which plant species deploy their economic strategies to benefit their nutrition (Figs 1, 6).

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Author contributions


LH and SF conceived the ideas and designed the methodology. LH, SF and CC collected the data. LH analyzed the data and led the writing of the manuscript. PK, DAW, CC and SF contributed critically to the drafts and gave final approval for publication.


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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Relationships of other soil N cycling processes with plant metabolic activity

Fig. S2 Relationships of plant N uptake with fine root production and of total plant N acquisition with rhizosphere priming.

Methods S1 Additional description of plant and soil C cycling and plant trait measurements, statistical analyses, and C and N budget computation.

Table S1 Sensitivity analysis of 1‰ variation in *B* for the estimation of plant N uptake.

Table S2 Description and coordinates of the variables in the ordination space of the redundancy analysis.

Table S3 Selection of multiple regression models relating soil N cycling properties to plant traits.

Table S4 Correlations of soil N cycling properties with plant economic traits and other ecosystem properties.

Table S5 Soil N cycling properties across species.

Table S6 Sensitivity analysis of bivariate regressions controlling for functional group differences.

Table S7 Plant N cycling properties across species.

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