



Season exerts stronger control than tree species on nitrogen cycling in the rhizosphere of young alder and oak forests

Novalia Kusumarini · Iseult Lynch ·
Liam R. Cox · Sami Ullah

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Abstract

Aims Understanding nitrogen (N) dynamics in young forest plantations is critical for predicting long-term soil fertility and greenhouse gas emissions.

Methods We investigated seasonal patterns of N transformation, extracellular enzyme activities, and soil respiration in rhizosphere soils of alder (*Alnus glutinosa*, N-fixing) and oak (*Quercus robur*, non-N-fixing) in a ~5-year-old mixed temperate plantation.

Results Seasonal dynamics strongly regulated N cycling, whereas species-specific effects were less pronounced. Gross N mineralization peaked in spring, closely associated with microbial biomass and N-acquiring enzyme activity, but declined

through summer and autumn. Gross nitrification showed a contrast pattern, resulting in a clear decoupling between mineralization and nitrification, likely reflecting stronger microbial immobilization and rhizosphere-mediated regulation in spring, supported by higher microbial biomass, which both weakened toward autumn. Net and gross N transformation differed markedly, with net mineralization highest in autumn, while net nitrification peaked in spring, and both were lowest in summer. Potential N₂O emissions also varied seasonally and between species, being highest in summer for alder and in spring for oak, and lowest in autumn for both. These findings highlight species-specific regulation in N cycling. Species comparisons revealed that alder soils, despite their N-fixing capacity, exhibited tighter microbial–plant coupling with higher N immobilization and lower nitrification, except in spring, while oak soils accumulated more NH₄⁺ and supported greater nitrification, reflecting contrasting rhizosphere strategies.

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N. Kusumarini (✉) · I. Lynch · S. Ullah (✉)
School of Geography, Earth and Environmental
Sciences, University of Birmingham, Edgbaston,
Birmingham B15 2TT, UK
e-mail: nxk120@student.bham.ac.uk; novakusuma@ub.ac.id

S. Ullah
e-mail: s.ullah@bham.ac.uk

N. Kusumarini
Faculty of Agriculture, Universitas Brawijaya, Malang,
Indonesia

L. R. Cox
School of Chemistry, University of Birmingham,
Edgbaston, Birmingham B15 2TT, UK

S. Ullah
Birmingham Institute of Forest Research, University
of Birmingham, Birmingham, UK

Conclusions Collectively, our findings demonstrate that seasonal dynamics, more than tree species identity, dominate rhizosphere N cycling in young plantations, with implications for nutrient retention, forest productivity, and climate feedback.

Highlights

1. Seasonal dynamic was the dominant control in the soil N cycle in the young forest.
2. Gross mineralization peaked in spring, driven by microbial biomass and N-acquiring enzymes.
3. Oak soil showed higher gross mineralization, nitrification, and NH_4^+ accumulation, reflecting a more rapid N cycle.
4. Species effects, though not yet significant, are emerging and are expected to strengthen as plantations mature.

Keywords Nitrogen dynamics · Rhizosphere N cycling · Forest productivity · Rhizosphere regulation · Soil fertility · Greenhouse gas emissions

Introduction

Nitrogen (N) is a fundamental nutrient regulating ecosystem productivity, yet its availability is often limited in forest soils. Nitrogen mineralization represents the primary pathway by which organic N in soil organic matter is converted into inorganic forms available to plants and microbes (Moreau et al. 2019; Sardar et al. 2023). These transformations are largely microbially mediated and are strongly influenced by plant species identity, particularly within the rhizosphere (Rengel and Marschner 2005; Henneron et al. 2020; Nakayama and Tatenno 2022). The rhizosphere is widely recognized as an N-cycling hotspot, where root exudates supply labile carbon that stimulates microbial metabolism, supports microbial interactions, and enhances extracellular enzyme production (Herman et al. 2006; Koranda et al. 2011; Coskun et al. 2017; Sun et al. 2021; Han et al. 2023). As a result, mineralization rates are typically higher in rhizosphere soil than in bulk soil (Herman et al. 2006; Rumeau et al. 2024). Symbiotic associations with mycorrhizal fungi and N-fixing bacteria further enhance N-cycling efficiency, expanding nutrient acquisition pathways and alleviating N limitation

for host plants (Perakis and Pett-Ridge 2019; Dellagi et al. 2020; Singh et al. 2022). This is particularly critical in young temperate plantations, where rapid tree establishment and growth place high demands on soil N. Nevertheless, N cycling in young forests is generally less rapid and less efficient than in mature forests, reflecting lower organic matter accumulation and developing microbial networks.

Comparing alder (*Alnus glutinosa*), which forms N-fixing root nodules, with oak (*Quercus robur*), which relies primarily on mycorrhizal associations (beneficial, mutualistic relationships between plant roots and fungi), provides a framework to understand how contrasting rhizosphere strategies regulate N cycling during the early stages of forest development. N-fixing species such as alder enrich forest soils by converting atmospheric N_2 into plant-available forms, such as ammonium (NH_4^+) via biological N fixation driven by symbiotic diazotrophic microbes (Perakis and Pett-Ridge 2019; Ortiz and Wolf 2024). This input can accelerate soil N accumulation, often leading to greater rates of nitrification relative to nitrate immobilization, thereby enhancing overall N mineralization efficiency (Siddique et al. 2008; Wang et al. 2010). However, this stimulation may also increase N_2O (a potent greenhouse gas) emissions to the atmosphere (Kou-Giesbrecht and Menge 2021; Xu et al. 2026). In contrast, non-N-fixing trees, such as oak, do not fix atmospheric N but instead depend largely on mycorrhizal symbioses to improve nutrient acquisition from existing soil pools (Dellagi et al. 2020; Pellitier et al. 2021). The ectomycorrhizal (ECM) associated trees often have efficient internal retained N cycling with slower net but greater gross N mineralization as their fungi capture amino acids following depolymerization, thus effectively short-circuiting inorganic N mineralization (Seyfried et al. 2023). However, N_2O emission do not necessarily coincide with peaks in N mineralization, as these peaks may be driven by NH_4^+ rather than NO_3^- , particularly in temperate oak forest (Yamulki and Morison 2017). Soil N_2O emissions are also strongly regulated by the climate, with higher moisture levels consistently enhancing N_2O production (Dick et al. 2006; Konda et al. 2010; Gundersen et al. 2012). Consequently, during the early growing season when soil moisture is typically elevated, N_2O emissions tend to rise in parallel with the increased denitrification activity (Kettunen et al. 2007). Rapid fluctuation in soil water content, especially across

intermediate moisture levels, has been identified as a key driver of elevated N_2O efflux both in spring and autumn (Mander et al. 2021). Overall, interspecific differences in N_2O emissions are largely governed by tree species effects on litter quality, litter layer structure, soluble C inputs via exudates, and soil moisture dynamics (Yamulki and Morison 2017).

Seasonality is one of the regulators of nutrient dynamics, particularly nitrogen (N) mineralization, as it strongly influences microbial activity and associated soil processes in deciduous forests. Both bacterial and fungal communities in the rhizosphere exhibit distinct seasonal shifts (Xu et al. 2023; Wan et al. 2025; Wu et al. 2025), largely shaped by environmental factors. Temperature plays a central role, with warmer conditions generally enhancing microbial abundance and activity, while colder conditions suppress them (Zhao et al. 2025). Similarly, soil moisture shapes microbial communities where moist soils support greater diversity and metabolic activity (Huo et al. 2024), while drought conditions reduce both microbial abundance and functional potential (Quiroga-González et al. 2025). These seasonal changes in microbial communities directly affect N mineralization (Xiao et al. 2022; Bruni et al. 2025). Consequently, N mineralization rates vary seasonally (Liu et al. 2017), often declining during periods of low temperature and limited precipitation, such as the late growing season or dormant season (Zhao et al. 2010; Yuan et al. 2023a), and increasing under favorable conditions of warmth and moisture during the growing season (Li et al. 2020; Feng et al. 2024). However, the magnitude and direction of these seasonal dynamics are strongly dependent on plant functional types and are further mediated by shifts in microbial communities and their functions throughout the growing season (Zhao et al. 2010; Li et al. 2020). Among the two species, alder (*Alnus spp.*) plays a strong role in shaping belowground processes because its symbiosis with *Frankia* spp. in root nodules enables active biological nitrogen fixation, thereby altering soil chemistry and restructuring microbial communities that control nutrient mineralization (Selmants et al. 2005; Krishna et al. 2019). However, N fixation is not constant throughout the year, where activity begins after bud break in early spring and peaks in summer as temperatures rise to meet tree N demands (Lee and Son 2005). Cooler temperatures suppress nitrogen fixation in *Alnus*, reducing both microbial activity and nitrogenase function

(Anderson and Markham 2021). These seasonal shifts in fixation capacity strongly influence the composition and functional potential of N-fixing microorganisms in both root nodules and the surrounding rhizosphere (Yuan et al. 2023b). In contrast, oak (*Quercus* spp.) relies on ectomycorrhizal (ECM) fungal associations rather than N-fixing symbioses. Nevertheless, seasonal variations also shape the oak rhizosphere, as ECM community composition and function are sensitive to seasonal changes in temperature, moisture, and plant phenology (Voříšková et al. 2014). Beyond differences in N acquisition, both alder and oak modulate microbial N cycling through seasonal changes in root exudation patterns, which influence microbial growth, activity, and nutrient transformations in the rhizosphere. Root exudate quantity and composition vary widely among plant species and are strongly regulated by environmental conditions such as nutrient availability and soil moisture (Kaiser et al. 2011; Daly and Hernandez-Ramirez 2020; Li et al. 2021; Seitz et al. 2022).

Although many studies have examined nitrogen (N) mineralization in temperate forests, there is still limited understanding of how contrasting planted young tree functional types, specifically N-fixing versus non-N-fixing species, regulate N cycling in the rhizosphere, particularly during the early stages of plantation development. To address this gap, the present study aimed to investigate seasonal dynamics of both gross and net N mineralization in the rhizosphere soils of a N-fixing species (alder, *Alnus glutinosa*) and a non-N-fixing species (oak, *Quercus robur*) in a young temperate plantation. While *Alnus* is widely recognized as an N-fixing tree through its symbiosis with *Frankia* in root nodules (Lee and Son 2005; Selmants et al. 2005; Voříšková et al. 2014), we did not directly quantify N-fixation rates in this study. Instead, we acknowledge its fixation capacity based on the presence of nodulation in alder roots (see photos in Fig S.1). In addition, we assess associated microbial activities, including extracellular enzyme activities and soil respiration, that underpin and regulate N cycling. We hypothesized that alder would exhibit higher gross nitrification and greater net N mineralization and nitrification, reflecting a more dynamic N cycle driven by N_2 fixation and elevated N availability. In contrast, oak was expected to show slightly higher gross mineralization and microbial immobilization,

thus lower nitrification, consistent with the internally retained N cycling typical of ectomycorrhizal systems. We further expected N mineralization to peak in spring and summer, followed by a decline in autumn as cooler temperatures and reduced tree and microbial N demands and microbial activity constrain soil processes.

Materials and methods

Study site and sampling method

Soil samples were collected in mid-spring, summer, and autumn of 2024 from a recently established (~5 years) polyculture plantation at the Birmingham Institute of Forest Research (BiFoR), Staffordshire, England (52°48'13.2" N, 2°17'46.1" W). Sampling was conducted in the rhizosphere by excavating soil directly adjacent to the main stem and carefully collecting the soil adhering to roots. The same trees were sampled across all three seasons to ensure consistency. Fresh samples were immediately passed through a 2-mm sieve and stored at 4 °C until subsequent N mineralization analyses. The soils were classified as silt loam, with a texture of $7.04 \pm 0.32\%$ clay, $56.05 \pm 1.47\%$ silt, and $36.91 \pm 1.75\%$ sand.

The soil samples were collected during mid-season for each sampling period, specifically during the first week of May, mid-July, and the end of October 2024. The annual average above-canopy temperature was 10.83 ± 0.3 °C (SE) with a maximum of 28.7 °C and a minimum of −4.3 °C. The annual rainfall averaged 676 ± 66 mm based on 2023 daily climate report (Rabbai et al. 2024).

Gross mineralization

Labeling and incubation

Gross N mineralization rates were determined using the ^{15}N isotope pool dilution method (Hart et al. 1994; Rumeau et al. 2024). Subsamples of 30 g fresh soil were placed into 500 mL mason jars fitted with rubber septa to allow for gas sampling. Labeling solutions containing either K^{15}NO_3 (98 atom% ^{15}N , Sigma-Aldrich) or $^{15}\text{NH}_4\text{Cl}$ (98 atom% ^{15}N , Sigma-Aldrich) were prepared at concentrations corresponding to 30% of the initial soil NH_4^+ or NO_3^- pools.

1 mL of label solution was applied to each soil sample through multiple injections to ensure even distribution. The jars were then sealed and incubated at 20 °C in the dark for 24 h. Following the incubation, the process was terminated by adding 150 mL of 1 M KCl. The suspension was then shaken at ~200 rpm for 1 h, centrifuged, and filtered through a 0.45 µm filter. 50 mL of extract was processed to determine the ^{15}N content in NH_4^+ and NO_3^- via the pool dilution method, and the remaining extract was analyzed for N- NH_4^+ and N- NO_3^- concentrations.

Simultaneously, 15 mL of headspace gas was sampled with a syringe immediately before jar closure and transferred to 12 mL pre-evacuated exetainer vials (Labco, UK). After 24 h of incubation, a second headspace gas sample was collected in the same manner. Gas samples were analyzed for CO_2 and N_2O concentrations using a gas chromatograph (GC; Agilent Technologies, USA) equipped with a micro electron capture detector (µECD) and a flame ionization detector (FID). The ^{15}N content of N_2O was quantified using a continuous-flow isotope ratio mass spectrometer (IRMS; Elementar Isoprime Precision, Elementar Analysensysteme GmbH, Hanau, Germany) coupled with a trace-gas preconcentrator and autosampler (isoFLOW GHG, Elementar Analysensysteme GmbH). Gas samples were purged into a He carrier stream, passed through CO_2 and H_2O scrubbers, and then entered a first liquid N_2 trap, isolating and cryofocusing, and separated from residual CO_2 on a gas chromatography column before entering the IRMS. N_2O isotopologues were measured as R45 and R46 ratios (mass-to-charge ratios of 45 and 46 relatives to 44) at an ion current of 600 µA. Instrument stability was verified by analyzing 10 N_2O reference samples before sample runs, achieving a $\delta^{15}\text{N}$ standard deviation of $\leq 0.05\text{‰}$.

The ^{15}N gas diffusion procedure

The diffusion procedure followed (Davidson et al. 1991; Hart et al. 1994) to isolate enriched NH_4^+ or NO_3^- pools onto acid traps for subsequent ^{15}N analysis using an elemental analyzer–isotope ratio mass spectrometer (EA-IRMS; Isoprime Precision, Elementar Analysensysteme GmbH, Germany). Acid traps were prepared from 8 mm Whatman 3 filter discs soaked with 10 µL of 2.5 M KHSO_4 and wrapped in PTFE tape. For NH_4^+ diffusion, 50 mL of

soil extract was amended with 0.2 g of MgO and an acid trap and incubated for 7 days, and daily gentle swirling to allow complete diffusion. The acid discs were then removed, dried in a desiccator for 24 h, encapsulated in tin capsules, and analyzed via EA-IRMS. To diffuse NO_3^- , NH_4^+ was first removed by performing the same procedure but discarding the initial acid traps. NO_3^- diffusion was then initiated by adding 100 μL of 30% Brij-35 and 0.4 g of Devarda's alloy, after which a fresh acid trap was inserted. Samples were incubated for an additional 7 days at room temperature with daily gentle shaking. The final acid traps were dried in a desiccator for 24 h, encapsulated, and analyzed for ^{15}N atom% using EA-IRMS.

Net mineralization

Net mineralization was determined by calculating the difference in NH_4^+ and NO_3^- concentrations between the start and end of incubation (Davidson et al. 1991). Fresh, sieved soil (30 g) was placed in mason jars fitted with rubber septa for gas sampling and incubated at 20 °C in the dark for 7 days. During incubation, 15 mL of headspace gas was collected daily to quantify soil respiration and potential N_2O emissions. Soil moisture was maintained by monitoring sample weight and replenishing with deionized water as needed. At the end of incubation, inorganic N (NH_4^+ and NO_3^-) was extracted with 1 M KCl and quantified colorimetrically in 96-well microplates (Sims et al. 1995). Net mineralization was then calculated as the increase in extractable inorganic N over the incubation period.

Soil nutrient analysis

Soil total organic carbon (TOC) and total nitrogen (TN) were quantified using an elemental analyzer (vario PYRO cube; Elementar Analysensysteme GmbH, Hanau, Germany) on air-dried soil. For each measurement, 28 mg of dried soil was encapsulated in a tin capsule before analysis. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined by extracting 5 g of fresh soil with 25 mL of 1 M KCl in 50 mL centrifuge tubes, shaking at 200 rpm for 1 h, centrifuging, and filtering through 0.45 μm membrane filters. Extracts were diluted 20-fold and analyzed using a Shimadzu TOC-L analyzer. Microbial biomass carbon (MBC) and nitrogen

(MBN) were estimated by the chloroform fumigation–extraction method (Brookes et al. 1985). DOC and TDN were extracted from fumigated and non-fumigated samples using the same procedure, and MBC and MBN were calculated using conversion factors of 0.45 and 0.54, respectively. Inorganic N (NH_4^+ and NO_3^-) concentrations were determined colorimetrically (Sims et al. 1995). Extracts were analyzed in clear 96-well plates, with absorbance measured spectrophotometrically at 650 nm for NH_4^+ and 540 nm for NO_3^- using a Tecan Spark® multimode microplate reader.

Enzyme assays

In this study, we quantified two groups of extracellular enzymes: hydrolytic and oxidative. Hydrolytic enzymes included β -glucosidase (BG), β -N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP), while oxidative enzymes included phenol oxidase (Phenox) and peroxidase (Perox).

Hydrolytic enzyme activities were determined using a fluorometric assay. Briefly, 1 g of fresh soil was suspended in 125 mL of 50 mM NaOAc buffer (pH 5.2) in a 250 mL beaker, sonicated for 2 min to homogenize, and stirred at ~300 rpm to maintain suspension. While stirring, 200 μL soil suspension was pipetted into sterile black 96-well plates containing 50 μL of specific substrate solution: 200 μM MUF- β -D-glucopyranoside for BG, 200 μM MUF- β -D-glucosaminidine for NAG, 200 μM MUF-phosphate disodium salt for AP, or 200 μM L-leucine-AMC for LAP. Reference standards consisted of 200 μL NaOAc buffer with 50 μL of either 4-methylumbelliferone (MUF) or 7-amino-4-methylcoumarin (AMC) across a 0.25–50 μM gradient. Blank standards received 200 μL soil suspension plus 50 μL MUF or AMC (5–50 μM). Blank control contained 200 μL soil suspension and 50 μL buffer, while negative controls contained 200 μL buffer and 50 μL substrate. Fluorescence was measured at 30 °C every 5 min over 1 h at an excitation wavelength of 365 nm and emission wavelength of 450 nm. Data from the first 10 min of pre-incubation were excluded, and fluorescence intensity over time was fitted with linear regression. Only regressions with $R^2 > 0.97$ were accepted, and slopes of the fitted lines were used to calculate enzyme activity.

Oxidative enzyme activities were determined using a colorimetric assay. Soil suspensions were prepared by sonicating 0.5 g of fresh soil in 125 mL of 50 mM NaOAc buffer (pH 5.2) for 2 min, followed by stirring at ~300 rpm. Substrates consisted of 25 mM L-DOPA for phenol oxidase and 25 mM L-DOPA plus 0.3% H₂O₂ for peroxidase. In clear 96-well plates, 200 µL soil suspension was combined with 50 µL L-DOPA (Phenox) or 50 µL L-DOPA plus 10 µL H₂O₂ (Perox). Negative controls contained buffer and substrate ($\pm$ H₂O₂), while blanks contained soil suspension and buffer ($\pm$ H₂O₂). The formation of dopachrome was measured spectrophotometrically at 460 nm at 30 °C. Absorbance was recorded every 5 min for 2 h (Perox) and periodically up to 48 h (Phenox). Enzyme activity was calculated as the slope of absorbance increase over time.

N transformation calculations

Gross mineralization and nitrification fluxes were calculated using the following equations:

$$M(\mu\text{g g}^{-1}\text{day}^{-1}) = \frac{[\text{NH}_4^+]_0 - [\text{NH}_4^+]_t}{t} \times \frac{\log\left(\frac{\text{APE}_0}{\text{APE}_t}\right)}{\log\left(\frac{[\text{NH}_4^+]_0}{[\text{NH}_4^+]_t}\right)} \quad (1)$$

$$C(\mu\text{g g}^{-1}\text{day}^{-1}) = M - \frac{[\text{NH}_4^+]_0 - [\text{NH}_4^+]_t}{t} \quad (2)$$

where M=gross mineralization rate, C=NH₄⁺ consumption rate, t=time elapsed, [NH₄⁺]=soil NH₄⁺ concentration (µg g⁻¹), and APE_t=¹⁵N atom percent excess in the NH₄⁺ pool at time t. Gross nitrification and NO₃⁻ consumption rates were determined using the same formula, substituting NH₄⁺ concentration and APE_t with NO₃⁻ concentration and the APE of the NO₃⁻ pool, respectively. The NH₄⁺ immobilization rate was calculated as the difference between the NH₄⁺ consumption rate and gross nitrification. NO₃⁻ immobilization was assumed to be equal to the gross NO₃⁻ consumption rate (Kirkham 1954).

CO₂ and N₂O emissions were calculated using the following equation:

$$\text{CO}_2 \text{ flux } (\mu\text{g g}^{-1}\text{day}^{-1}) = \frac{([\text{CO}_2]_t - [\text{CO}_2]_0) \times H}{m \times t} \quad (3)$$

where [CO₂]_t=concentration of CO₂ in µg C L⁻¹ in the vial at time t, H=headspace volume in

L, m=mass of dry soil (g), and t=time elapsed (h). N₂O flux was calculated similarly by substituting the concentration of N₂O (in µg N L⁻¹) into the equation. N₂O emissions from nitrification and denitrification were calculated following the methods described in (Sgouridis et al. 2023).

To assess microbial activity, the microbial metabolic quotient (qCO₂) was calculated by dividing microbial respiration by the microbial biomass C (Spohn and Chodak 2015).

Statistical analysis

Statistical analyses were conducted using RStudio (version 2024.04.2+764; R Core Team 2017). Before analysis, data were screened for normality using the Shapiro–Wilk test and for linearity by examining scatterplots and residuals versus fitted values. Variables that deviated from normality were log-transformed to meet the model assumption. A linear mixed model (LMM) was used to assess the effects of soil sources (alder and oak rhizosphere soil) and seasons on the responses. This analysis was conducted using the lmer function from the lme4 package. Because the same plots and trees were sampled repeatedly across seasons, the plot was included as a random effect to account for repeated measurements. Correlations among variables were examined using Spearman correlation tests and R-squared coefficients.

Results

Soil biogeochemical properties

Most soil properties exhibited significant seasonal variation ($p < 0.05$), except for total nitrogen (TN) and total organic carbon (TOC) pools, which remained stable across seasons, but no significant differences between alder and oak rhizosphere soils (Table S1). Seasonal patterns, however, were not consistent across soil parameters, except in available N (N–NH₄⁺ and N–NO₃⁻). On average, N–NH₄⁺ concentrations were lowest in spring and increased progressively throughout the growing season, with values 60% higher in summer and 209% higher in autumn relative to spring (Table 1). N–NO₃⁻ followed a similar seasonal trajectory, but species-specific differences were evident in spring, where oak rhizosphere

Table 1 Soil nutrient content and microbial biomass in Alder and Oak rhizosphere soils. Values represent mean $\pm$ SE. Different letters following the numerical values indicate significant differences among treatments ($p < 0.05$)

	Alder			Oak		
	Spring	Summer	Autumn	Spring	Summer	Autumn
TOC (%)	1.82 $\pm$ 0.09 ^a	1.88 $\pm$ 0.06 ^a	2.04 $\pm$ 0.09 ^a	1.85 $\pm$ 0.14 ^a	2.09 $\pm$ 0.03 ^a	1.87 $\pm$ 0.06 ^a
TN (%)	0.16 $\pm$ 0.009 ^a	0.17 $\pm$ 0.006 ^a	0.18 $\pm$ 0.007 ^a	0.15 $\pm$ 0.009 ^a	0.18 $\pm$ 0.003 ^a	0.16 $\pm$ 0.004 ^a
C:N	11.55 $\pm$ 0.35 ^a	11.37 $\pm$ 0.23 ^a	11.20 $\pm$ 0.10 ^a	11.93 $\pm$ 0.23 ^a	11.77 $\pm$ 0.14 ^a	11.66 $\pm$ 0.13 ^a
DOC ($\mu\text{g g}^{-1}$)	71.08 $\pm$ 3.16 ^a	69.33 $\pm$ 4.59 ^a	133.12 $\pm$ 16.51 ^b	84.85 $\pm$ 6.33 ^a	72.55 $\pm$ 4.59 ^a	93.81 $\pm$ 2.44 ^a
TDN ($\mu\text{g g}^{-1}$)	14.97 $\pm$ 0.72 ^a	25.28 $\pm$ 1.41 ^c	24.89 $\pm$ 0.69 ^{bc}	18.05 $\pm$ 1.60 ^{ab}	25.28 $\pm$ 1.71 ^c	28.23 $\pm$ 1.59 ^c
DOC:TDN	4.75 $\pm$ 0.07 ^c	2.77 $\pm$ 0.11 ^a	5.37 $\pm$ 0.67 ^c	4.73 $\pm$ 0.10 ^{bc}	2.88 $\pm$ 0.03 ^a	3.35 $\pm$ 0.10 ^{ab}
N-NH ₄ ⁺ ($\mu\text{g g}^{-1}$)	3.07 $\pm$ 0.27 ^a	6.78 $\pm$ 0.25 ^b	9.51 $\pm$ 0.22 ^c	4.05 $\pm$ 0.42 ^a	7.00 $\pm$ 0.24 ^b	12.50 $\pm$ 0.48 ^d
N-NO ₃ ⁻ ($\mu\text{g g}^{-1}$)	7.07 $\pm$ 0.96 ^a	9.41 $\pm$ 0.70 ^a	19.88 $\pm$ 1.69 ^b	3.29 $\pm$ 0.45 ^a	9.47 $\pm$ 1.62 ^a	19.84 $\pm$ 2.05
MBC ($\mu\text{g g}^{-1}$)	71.63 $\pm$ 3.61 ^a	61.02 $\pm$ 1.84 ^a	53.30 $\pm$ 8.27 ^a	68.14 $\pm$ 5.19 ^a	62.75 $\pm$ 3.86 ^a	62.04 $\pm$ 3.64 ^a
MBN ($\mu\text{g g}^{-1}$)	26.77 $\pm$ 1.14 ^b	18.08 $\pm$ 1.43 ^b	18.67 $\pm$ 1.00 ^a	27.57 $\pm$ 1.82 ^a	18.26 $\pm$ 1.43 ^a	17.53 $\pm$ 1.10 ^a
MBC:MBN	2.67 $\pm$ 0.13 ^a	3.40 $\pm$ 0.13 ^a	2.86 $\pm$ 0.46 ^a	2.46 $\pm$ 0.10 ^a	3.47 $\pm$ 0.10 ^a	3.54 $\pm$ 0.07 ^a

soil contained 115% less N-NO₃⁻ than alder, although concentrations were similar in summer and autumn (Table 1). These patterns suggest a seasonal buildup of inorganic N, likely driven by mineralization events during the growing season.

Microbial biomass exhibited the opposite seasonal pattern to available nitrogen. MBC and MBN were highest in spring and declined toward autumn, both in alder and oak rhizosphere soils (Table 1). Despite this overall decline, alder soils maintained 34% higher MBC and 43% higher MBN in autumn compared to spring, while oak soils showed increases of 10% and 57%, respectively. The seasonal reduction in microbial biomass, particularly MBN, was in line with the accumulation of available N both in alder and oak soils, indicating a shift from strong microbial immobilization in spring toward N mineralization later in the growing season. Consistent with this, MBN was negatively correlated with N-NH₄⁺ ($r = -0.70$, $p < 0.001$) and N-NO₃⁻ ($r = -0.55$, $p = 0.002$) (Table S.2), supporting the view that microbial uptake constrained N availability in spring, whereas microbial turnover likely contributed to N release in summer and autumn.

Soil microbial activities

Soil microbial activity, assessed through extracellular enzyme activities, soil respiration, and metabolic quotient ($q\text{CO}_2$), was not significantly influenced by soil source (alder vs. oak rhizosphere) but showed strong

seasonal dynamics. However, for certain parameters, the soil source effect became apparent only through its interaction with season, particularly in BG and AP activities, where seasonal shifts modulated species-specific differences. Overall, microbial activity tended to peak in summer, with most enzyme activities and soil respiration reaching their highest values during this season. Exceptions were observed in oak soils, where NAG, AP, Phenox, soil respiration, and $q\text{CO}_2$ were higher in spring than in summer. In autumn, all measured microbial responses declined to their lowest levels (Fig. 1). Across soil sources, oak soils generally exhibited higher activities of C-acquiring enzymes (BG, Phenox, Perox) than alder soils (Fig. 1), whereas N-acquiring enzyme activities (NAG and LAP) showed no consistent seasonal trend. In contrast, soil respiration and $q\text{CO}_2$ were consistently higher in alder soils, particularly in summer and autumn, averaging 65% and 117%, respectively, above oak soils during these seasons, likely reflecting the contribution of root nodules. The higher $q\text{CO}_2$ observed in alder soils indicates greater microbial respiration per unit biomass, likely driven by alder root nodules, and is generally associated with reduced carbon use efficiency (CUE). Across all samples, soil respiration was positively correlated with enzyme activities ($r = 0.41$ – 0.61 , all $p < 0.05$), while $q\text{CO}_2$ performed a moderate positive correlation with enzyme activities ($r = 0.37$ – 0.58 , $p < 0.05$) (Fig. S.3), underscoring the strong linkage between extracellular enzyme activity and microbial metabolic processes in the rhizosphere.

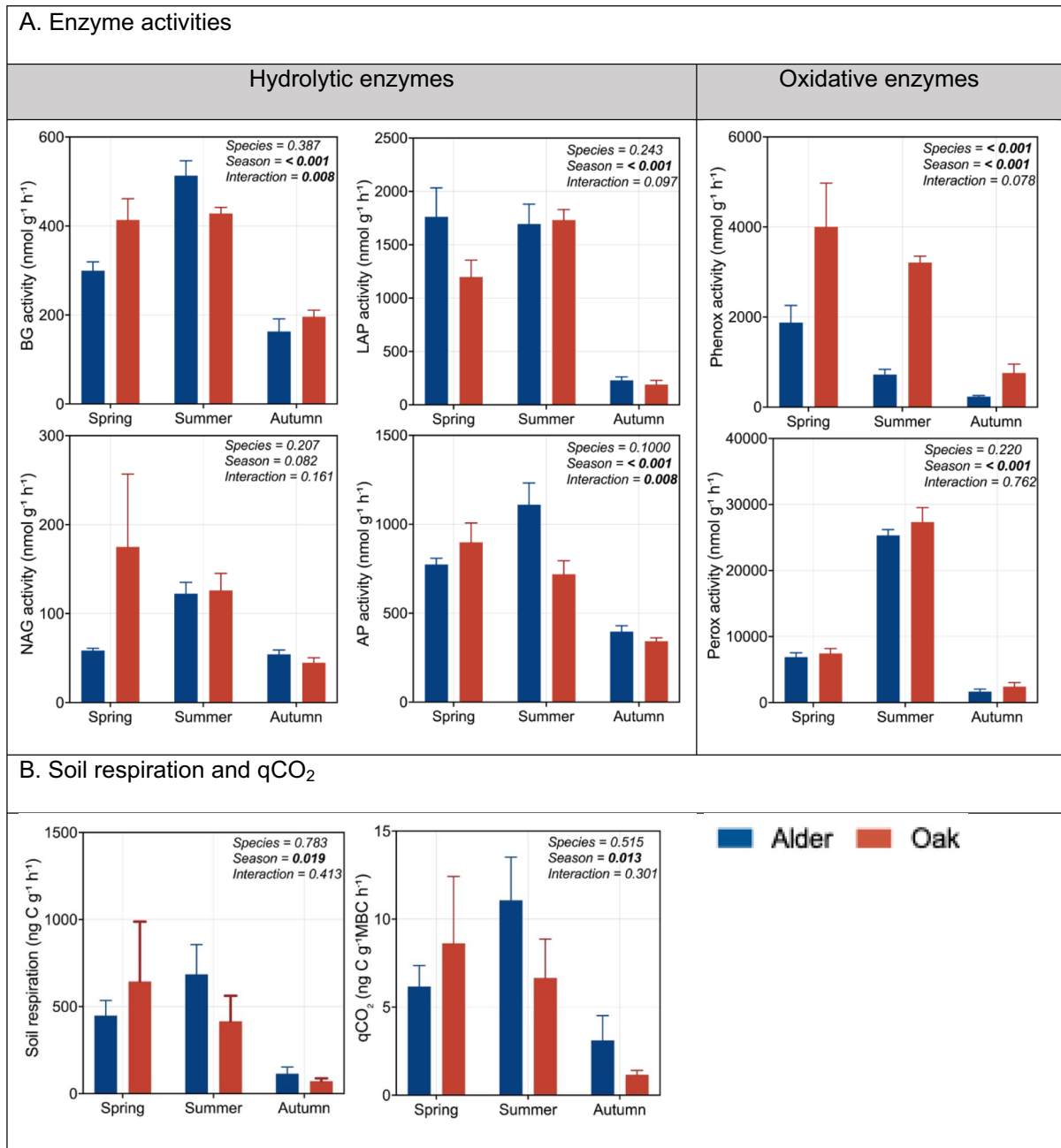


Fig. 1 **A** Enzyme activities and **(B)** soil respiration in Alder and Oak rhizosphere soil across the growing seasons. Values represent means $\pm$ standard error ($n=5$). P-values indicating

the effects of species, season, or their interaction are shown in the top of each panel

Soil nitrogen transformation

Gross nitrogen mineralization rates

Gross mineralization and nitrification rates varied significantly across seasons ($p < 0.001$), while only gross nitrification differed between alder and oak soils ($p = 0.039$) (Table S.2). Although it was not statistically significant, gross mineralization tended to be higher in oak than in alder soils, except in autumn, when it was 6.4% lower in oak. Seasonal dynamics were pronounced, with gross mineralization peaking in spring and then declining sharply, by 4.5-fold in summer and 5.3-fold in autumn relative to spring (Table 2), particularly in alder. In contrast, gross nitrification was lowest in spring and increased progressively over the growing season, rising by 10% in alder soils and 270% in oak soils in summer, and reaching its maximum in autumn with increases of 90% and 841% in alder and oak soils, respectively (Table 2). Oak soils consistently exhibited higher gross nitrification rates than alder soils. These contrasting seasonal patterns of gross mineralization and nitrification were reflected in their associations with microbial biomass. Both MBC and MBN peaked in spring and declined gradually thereafter. Gross mineralization was strongly and positively correlated with MBN ($r = 0.72$, $p < 0.001$; Fig. S.4), indicating that higher

microbial biomass nitrogen supported greater mineralization activity. In contrast, gross nitrification exhibited a weak, non-significant negative correlation with MBN ($r = -0.26$, $p = 0.171$; Fig. S.4), indicating that nitrification dynamics were less directly linked to microbial biomass. Additionally, correlations with enzyme activities revealed that only phenox was strongly and positively associated with gross mineralization ($r = 0.66$, $p < 0.001$; Fig. S.4), while other enzymes, including the N-acquiring enzymes NAG ($r = 0.36$) and LAP ($r = 0.43$), showed weaker positive correlations (Fig. S.4). Gross nitrification, by contrast, exhibited weak negative correlations with all measured enzyme activities. Moreover, Gross nitrification was strongly and positively correlated with both NH_4^+ concentration ($r = 0.60$, $p < 0.001$), reflecting its role as the primary substrate, and NO_3^- concentration ($r = 0.57$, $p = 0.001$) (Fig. S.5), consistent with its production as the end product. This relationship aligns with the seasonal trend, where the accumulation of NH_4^+ in summer and autumn likely fueled the progressive increase in gross nitrification rates and the corresponding rise in NO_3^- concentrations.

The high gross mineralization rate observed in spring did not translate into elevated NH_4^+ concentrations during the same season, likely due to concurrent high immobilization. Gross mineralization and immobilization were strongly and positively correlated

Table 2 Nitrogen transformation in Alder and Oak rhizosphere soils. Values represent mean $\pm$ SE. Different letters following the numerical values indicate significant differences among treatments ($p < 0.05$)

	Alder			Oak		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Gross N mineralization ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	22.41 $\pm$ 1.44 ^b	4.99 $\pm$ 0.53 ^a	4.21 $\pm$ 0.37 ^a	22.60 $\pm$ 0.44 ^b	5.79 $\pm$ 0.34 ^a	3.94 $\pm$ 0.52 ^a
Gross nitrification ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	1.68 $\pm$ 0.25 ^a	1.84 $\pm$ 0.52 ^a	3.19 $\pm$ 1.44 ^a	0.80 $\pm$ 0.12 ^a	2.96 $\pm$ 0.55 ^a	7.53 $\pm$ 0.89 ^b
NH_4^+ consumption ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	20.09 $\pm$ 1.56 ^b	8.11 $\pm$ 0.66 ^a	6.38 $\pm$ 0.88 ^a	19.64 $\pm$ 0.43 ^b	9.21 $\pm$ 0.21 ^a	5.09 $\pm$ 0.42 ^a
NO_3^- consumption ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	18.95 $\pm$ 1.59 ^c	2.91 $\pm$ 0.72 ^{ab}	-4.27 $\pm$ 1.56 ^a	9.72 $\pm$ 1.16 ^b	4.98 $\pm$ 0.71 ^b	6.46 $\pm$ 3.54 ^b
Immobilization ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	18.42 $\pm$ 1.54 ^c	6.27 $\pm$ 0.43 ^b	3.19 $\pm$ 1.82 ^b	18.84 $\pm$ 0.44 ^c	6.25 $\pm$ 0.62 ^b	-2.44 $\pm$ 1.07 ^a
Net N mineralization ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	0.70 $\pm$ 0.04 ^b	0.18 $\pm$ 0.03 ^a	1.60 $\pm$ 0.08 ^d	0.66 $\pm$ 0.03 ^b	0.14 $\pm$ 0.03 ^a	1.31 $\pm$ 0.09 ^c
Net nitrification ($\mu\text{g g}^{-1} \text{ day}^{-1}$)	2.62 $\pm$ 0.23 ^d	0.46 $\pm$ 0.11 ^a	0.73 $\pm$ 0.18 ^{ab}	1.78 $\pm$ 0.30 ^{cd}	0.35 $\pm$ 0.13 ^a	1.54 $\pm$ 0.20 ^{bc}
N_2O emission potential ($\text{ng g}^{-1} \text{ day}^{-1}$)	4.18 $\pm$ 0.88 ^a	12.95 $\pm$ 2.75 ^b	0.18 $\pm$ 0.04 ^a	19.94 $\pm$ 2.93 ^b	4.53 $\pm$ 0.56 ^a	0.18 $\pm$ 0.08 ^a
N_2O emission from nitrification ($\text{ng g}^{-1} \text{ day}^{-1}$)	0.07 $\pm$ 0.01 ^a	0.13 $\pm$ 0.02 ^{abc}	0.22 $\pm$ 0.14 ^c	0.05 $\pm$ 0.1 ^a	0.20 $\pm$ 0.02 ^{bc}	0.11 $\pm$ 0.01 ^{ab}
N_2O emission from denitrification ($\text{ng g}^{-1} \text{ day}^{-1}$)	0.03 $\pm$ 0.005 ^a	0.09 $\pm$ 0.039 ^a	0.14 $\pm$ 0.099 ^a	0.02 $\pm$ 0.001 ^a	0.26 $\pm$ 0.151 ^a	0.08 $\pm$ 0.028 ^a

($r=0.90$, $p<0.001$, Fig. S.7), indicating that much of the NH_4^+ released through mineralization was rapidly assimilated into microbial biomass. Immobilization rates varied significantly with season ($p<0.001$, Table S.2), peaking in spring and declining progressively over summer and autumn. The negative immobilization in autumn, particularly in oak rhizosphere soil, indicated net microbial N release rather than uptake. This seasonal decline in immobilization likely reflected the dynamics of MBN, which was highest in spring and decreased throughout the growing season, as supported by the strong positive correlation between immobilization rate and MBN ($r=0.67$, $p<0.001$, Fig. S.4). As immobilization weakened later in the season, NH_4^+ accumulated in the soil, providing substrate for enhanced nitrification in summer and autumn. The reduction of microbial N immobilization may be partly explained by the input of fresh litter, which increased N availability and likely alleviated microbial N limitation, reducing the need to immobilize inorganic N. To aid interpretation, a schematic figure summarizing the N cycle is presented in Fig. 2.

Net nitrogen mineralization rates

Net mineralization rates varied significantly with both species ($p=0.044$) and season ($p<0.001$) (Table S.2). In contrast to gross mineralization, which peaked in spring, net mineralization reached its highest values in autumn, being 130% higher in alder soils and 100% higher in oak soils compared to spring (Table 2). By autumn, however, both gross mineralization and immobilization were at their lowest, with immobilization rates in oak even becoming negative, resulting in greater net mineralization as little of the produced NH_4^+ was recaptured. Consistent with this, net mineralization was weakly and negatively correlated with gross mineralization ($r=-0.28$, $p=0.025$, Fig. S.7) but strongly and negatively correlated with microbial activity ($r=-0.52$ to -0.86 , $p<0.004$, Fig. S.4), underscoring the central role of microbial processes in regulating mineralization dynamics.

Net nitrification, in contrast, was highest in spring, despite gross nitrification being lowest at that time. This pattern may reflect minimal NO_3^- consumption in spring due to low plant uptake and

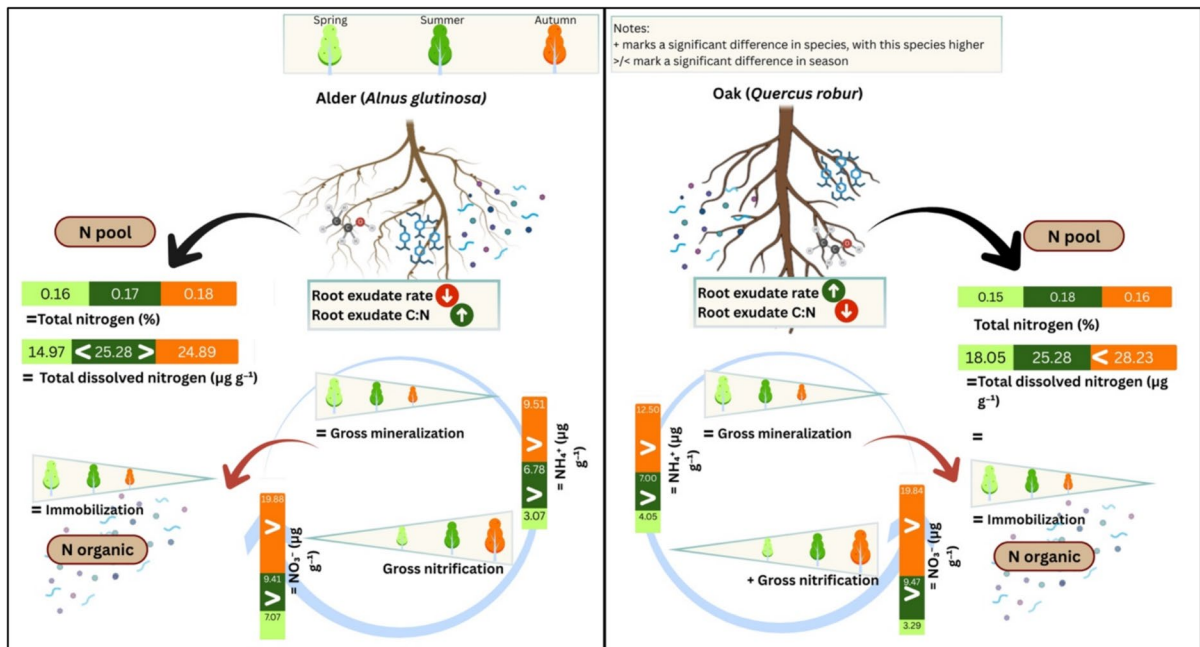


Fig. 2 Schematic representation of N cycling processes in young alder and oak plantations across seasons. A (+) indicates a significant species difference, with the marked species showing a higher value than the other, while (=) denotes no

significant species difference. Seasonal colors indicate sampling periods: light green for spring, green for summer, and orange for autumn

reduced denitrification activity relative to later in the season. Supporting this interpretation, net nitrification was positively correlated with N_2O emissions from both nitrification ($r=0.65$, $p<0.001$) and denitrification ($r=0.50$, $p=0.005$) (Fig. S.7). Although seasonal patterns did not align closely, net nitrification still showed a moderate correlation with gross nitrification ($r=0.41$, $p=0.319$, Fig. S7). The lowest net mineralization and nitrification rates occurred in summer, likely due to intensified plant N uptake during peak photosynthetic activity and enhanced N_2O emissions that also peaked in summer (Table 2).

N₂O emission rate

Potential N_2O emissions varied significantly across seasons ($p<0.001$), but the seasonal patterns differed between species (Table S.2). In alder soils, emissions peaked in summer, reaching values 73-fold higher than those measured in autumn. In contrast, oak soils exhibited maximum emissions in spring, which were also 112-fold higher than autumn levels. For both species, autumn represented the period of lowest potential N_2O emissions (Table 2).

Gross N_2O emission rates from both nitrification and denitrification did not differ significantly between alder and oak soils ($p>0.05$). Across seasons, only N_2O emissions derived from nitrification varied significantly ($p<0.001$) (Table S.2). In both species, N_2O emission from nitrification were lowest in spring and increased progressively thereafter, peaking in autumn for alder soils and in summer for oak soils. N_2O emission from denitrification showed a broadly similar seasonal pattern, although differences were not statistically significant. While net nitrification peaked in spring and declined to its lowest values in summer, gross N_2O emissions from nitrification and denitrification were lowest in spring. They increased later in the season, suggesting that only a fraction of the nitrified N was released as N_2O early in the season. In contrast, conditions in summer and autumn lead to greater gaseous losses.

Discussion

The concentration of available N (NH_4^+ and NO_3^-) increased over the growing season, whereas TOC and

TN pools remained stable. This pattern suggests that seasonal changes in microbial processes were the primary drivers of N mineralization, likely due to temperature increases and tree N demands (Schimel and Bennett 2004; Bagherzadeh et al. 2008). In both alder and oak rhizospheres, DOC concentrations peaked in autumn, likely reflecting the seasonal pulse of soluble carbon leached from newly deposited litter (Hensgens et al. 2020). However, this increase in DOC did not translate into higher microbial biomass (MBC), which is consistent with observations that microbial abundance often declines in autumn as falling temperatures constrain microbial growth and activity (Lepcha and Devi 2020; Kim et al. 2022). Furthermore, the small magnitude of MBC relative to TOC is expected, as microbial biomass constitutes only a minor fraction of total organic carbon (Anderson and Domsch 1980).

Despite the high N demand by trees in spring, N mineralization remained low because much of the plant N requirement was met through internal N remobilization from stored reserves (Bausenwein et al. 2001; Byne and Ryser 2020). As leaves reached full expansion later in the season, trees increasingly relied on soil N uptake, with N availability also regulated by plant strategies to sustain their nutrient demand. For example, fast-growing species can efficiently acquire and retain bioavailable N to match their rapid growth (Huang et al. 2023). By autumn, however, NH_4^+ concentrations increased, likely due to reduced microbial immobilization and the release of N from residue decomposition (Booth et al. 2005; Gao et al. 2021). This accumulation reflects a lag between microbial cell death and the subsequent mineralization of microbial biomass by other decomposers, during which released N is temporarily retained as NH_4^+ in the soil (Gao et al. 2021). Because N mineralization is an enzymatic process (Norton and Schimel 2011), the strong correlations observed between enzyme activities and mineralization rates in this study underscore the microbial control over net N mineralization. Notably, oak soils exhibited generally higher enzyme activities than alder soils. Unlike alder, which can satisfy part of its N demand through symbiotic N_2 fixation, oak depends entirely on ectomycorrhizal (ECM) associations to acquire N from organic matter. ECM fungi and their associated microbes secrete a suite of oxidative and hydrolytic enzymes that depolymerize complex organic

substrates, thereby releasing amino acids and other low-molecular-weight compounds available for host uptake (Talbot and Treseder 2010; Phillips et al. 2013; Averill et al. 2014). The pronounced spring peaks in N-acquiring enzyme activities and soil respiration in oak soils indicate an early-season investment in nutrient acquisition, reflecting a strategy to mobilize organic N when soil inorganic N availability is still low (Kunito et al. 2024). In contrast, alder soils showed higher total soil respiration, likely driven by greater autotrophic (root) respiration associated with N₂-fixing symbioses, whereas fungi in non-N₂-fixing (oak) soils incorporated more substrate-derived C into microbial biomass, reflecting distinct C–N coupling strategies (Luo et al. 2016). These differences in enzymatic activity and microbial nutrient acquisition strategies might also be reflected in patterns of inorganic nitrogen dynamics, particularly the accumulation of NH₄⁺ in alder and oak soils across seasons.

Across species, NH₄⁺ concentrations were consistently higher in oak soils than in alder soils, averaging 32% higher in spring and autumn and 3% higher in summer. This difference may also be associated with the larger dissolved nitrogen (DN) pool in oak soils, which was 21% higher in spring and 13% higher in autumn relative to alder. Because DN serves as a readily available substrate for microbial mineralization to NH₄⁺, the greater DN pool in oak soils likely contributed to the higher NH₄⁺ concentrations observed (Schimel and Bennett 2004; Jones et al. 2004; Ghani et al. 2007). Moreover, the C:N ratio of the soil was relatively low, which translates to high N mineralization given the availability of the substrate (Chiriac et al. 2025). In contrast, although alder can fix atmospheric N₂, the ammonia generated by this process is rapidly assimilated within the plant via glutamine synthetase rather than being released into the soil (Huss-Danell 1997). Furthermore, much of the NH₄⁺ produced through mineralization in alder soils is quickly immobilized by the rhizosphere microbial community, intensifying microbial–plant competition for inorganic N (Kuzyakov and Xu 2013; L'Espérance et al. 2024). This mechanism is consistent with the slightly higher immobilization rates observed in alder soils, particularly in autumn (Fig. 2). Moreover, symbiotic interactions with *Frankia* may further influence alder N dynamics, as studies have shown that in non-leguminous actinorhizal nodules, nitrate (NO₃[−]) concentrations can exceed those in

rhizosphere soil, reflecting differential allocation of available N between symbionts and rhizosphere pools (Yuan et al. 2023b). Unlike alder, oak does not form symbioses with N-fixing bacteria but instead relies on ectomycorrhizal (ECM) fungi to acquire N from soil (Morris et al. 2008). ECM fungi secrete hydrolytic and oxidative extracellular enzymes that degrade soil organic matter (SOM), thereby releasing N-bearing compounds for host uptake, such as amino acids released via depolymerization (Phillips et al. 2013). Consistent with this strategy, our results showed that both hydrolytic and oxidative enzyme activities were relatively higher in oak soils than in alder soils, supporting enhanced gross mineralization. Moreover, the greater abundance and activity of ECM fungi may also contribute to elevated NH₄⁺ availability in oak soils (Lei et al. 2022; Yan et al. 2025). This elevated inorganic N availability may promote fungal processing of organic matter through enhanced extracellular enzyme activity, characteristic of ectomycorrhizal (ECM) associations. Such enzyme-mediated depolymerization potentially facilitates the formation of microbial by-products that can become mineral-associated organic carbon (MAOC) (Averill et al. 2014; Wang et al. 2019).

Gross N mineralization peaked in spring and declined through summer and autumn, apparent asynchrony with plant phenology, consistent with findings by (Van Der Krift and Berendse 2001). This pattern is most likely explained by the higher microbial biomass observed in spring (MBC and MBN) in both alder and oak soils, as N mineralization strongly depends on microbial biomass (Schimel and Bennett 2004; Bruni et al. 2025). In addition, the relatively low MBC:MBN ratios in our soils suggest N-enriched microbial biomass, possibly resulting from atmospheric N deposition. Such N enrichment is known to enhance N mineralization (Verma and Sagar 2020) and increase soil N availability (Li et al. 2016). Spring typically provides optimal conditions of soil temperature and moisture that stimulate microbial growth (Schnecker et al. 2023), while microbial biomass is further regulated by climatic factors such as temperature, soil moisture, pH, and C:N ratio (Sarkar et al. 2024). Although many studies in temperate forests report higher microbial biomass in autumn (Babur et al. 2021; Yin et al. 2022), seasonal patterns are influenced by latitude: mid-latitude sites, such as the UK, where this study was conducted, often show

spring maxima, whereas low- and high-latitude forests more often peak in autumn (Zhao et al. 2022). In summer, by contrast, high temperatures coupled with reduced soil moisture suppress microbial activity and biomass (Baldrian et al. 2010; Brockett et al. 2012; Huang et al. 2018). Supporting this, N-acquiring enzyme activities (NAG and LAP), which are important drivers of N mineralization (Norton and Schimel 2011), were also elevated in spring, likely reflecting seasonal shifts in microbial community composition (He et al. 2023). Additionally, the temperature and higher water content generally enhanced soil N mineralization, particularly after soil freezing in winter (Zhou et al. 2011; Hosokawa et al. 2017).

However, the high gross mineralization in spring was not accompanied by elevated NH_4^+ concentrations, as mineralized N was rapidly immobilized by the abundant microbial biomass (Schimel and Bennett 2004; Booth et al. 2005; Gannett et al. 2024). This interpretation is consistent with our finding of the highest immobilization rates in spring, which decreased through season and reach negative value in autumn, specifically in oak rhizosphere soil. Net mineralization did not follow the same seasonal trajectory as gross mineralization, instead reaching its maximum in autumn. This discrepancy is likely due to rapid immobilization in spring, while the downregulation of N-acquiring enzymes and reduced microbial biomass in autumn allowed more mineralized N to remain available in the soil. Thus, the relationship between N-acquiring enzyme activity and net mineralization appears decoupled and dependent on microbial N demand (Liu et al. 2021). Importantly, these seasonal patterns were further modulated by species effects. Alder soils exhibited stronger immobilization and tighter microbial–plant coupling, enhancing N retention and soil N use efficiency (Gao et al. 2021). In contrast, oak soils, with higher gross mineralization and lower immobilization, showed greater NH_4^+ accumulation, reflecting a more open N cycle. This elevated inorganic N availability may promote fungal processing of organic matter and contribute to the formation of mineral-associated organic carbon (Wang et al. 2019).

Gross nitrification exhibited an opposite seasonal trend to gross mineralization, peaking in autumn. This decoupling between the two processes is commonly observed in young, regenerating forests, where N cycling tends to be more conservative to retain N for plant growth (Figueiredo et al. 2019). In our study site, a ~5-year-old mixed plantation, this pattern

likely reflects the closed N cycle typical of early successional stands. Importantly, nitrification is known to follow a log-linear relationship with mineralization; it increases sharply at low mineralization rates but responds only weakly when mineralization is high (Booth et al. 2005). This non-linear relationship provides further explanation for the divergence between our gross mineralization and nitrification data. Additionally, nitrification is strongly linked to heterotrophic microbial activity and fuelled by DOC availability (Ward and Bradford 2025). In our study, DOC concentrations were 9% lower in spring than in autumn; however, high microbial immobilization in spring likely suppressed nitrification by reducing the NH_4^+ available as substrate (Booth et al. 2005; Vázquez et al. 2020). Finally, because our study focused on rhizosphere soils, root activity may also have played a role. Root exudates are known to regulate nitrification, including through the release of biological nitrification inhibitors (BNIs), which conserve NH_4^+ in the rhizosphere to sustain plant N demand (Sun et al. 2016; Karwat et al. 2018). Across species, oak consistently showed higher gross nitrification rates than alder, likely reflecting its greater NH_4^+ pool and weaker microbial immobilization, while alder's tighter microbial–plant coupling constrained the availability of substrate for nitrifiers despite similar seasonal trajectories.

Gross nitrification was closely coupled with soil NO_3^- concentrations, as higher gross nitrification directly translated into greater NO_3^- accumulation, given that NO_3^- is the immediate end product of this process (Schimel and Bennett 2004; Booth et al. 2005). In contrast, net nitrification did not follow this trend because it is shaped not only by production but also by multiple loss pathways, including denitrification (Norton and Stark 2011). In our study, N_2O emissions both from nitrification and denitrification were decoupled from net nitrification, with the lowest net nitrification observed in summer, coinciding with the highest N_2O emissions. This suggests that nitrification is not fully efficient, as a portion of the nitrite is lost during the process (Malyan et al. 2025). Additionally, in the rhizosphere, strong competition between roots and microbes for NO_3^- further constrained its accumulation (Kuzakov and Xu 2013). However, patterns of NO_3^- consumption in our data did not consistently align with either net nitrification or overall, NO_3^- pool dynamics, reflecting the

complexity of competing processes controlling inorganic N availability in the rhizosphere.

Conclusion

This study demonstrates that nitrogen cycling in young temperate plantations is primarily regulated by seasonal dynamics, with species effects emerging subtly but likely to strengthen as stands mature. Gross N mineralization peaked in spring, coinciding with high microbial biomass (MBC and MBN) and elevated N-acquiring enzyme activities, and declined steadily toward autumn. In contrast, gross nitrification was lowest in spring and increased toward autumn, accompanied by immobilization, resulting in higher NH_4^+ and NO_3^- concentrations. Potential N_2O emissions also exhibited clear seasonal patterns, peaking in spring for oak and in summer for alder, and reaching their minimum in autumn for both species.

Although species differences were not statistically significant, consistent trends suggest functional divergence in rhizosphere N cycling. Oak soils showed higher gross mineralization and nitrification rates and greater NH_4^+ accumulation, reflecting a more open N cycle and rapid inorganic N turnover. In contrast, alder soils exhibited higher net mineralization and nitrification (except in autumn) and stronger microbial immobilization, indicating tighter microbial–plant coupling and more efficient N retention. Together, our results suggest that while seasonality exerts the dominant influence on N cycling in early stand development, tree species differences are already detectable and subtly steer the pathways and efficiency of N transformations matching tree phenological and N acquisition strategies. These findings suggest that the selection of tree species in temperate plantations can influence soil N turnover and retention, with potential consequences for nutrient use efficiency, greenhouse gas mitigation, and long-term soil fertility management.

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Data Availability Data available upon request.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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