

# Nitrogen leaching and soil nutrient supply vary spatially within a temperate tree-based intercropping system but are unaffected by tree root pruning

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## Research Article

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# Abstract

Well-designed temperate tree-based intercropping (TBI) systems can enhance soil nutrient cycling compared to conventional agricultural systems. To improve the TBI designs and their subsequent wide-scale adoption, greater understanding is required regarding the extent to which widely-spaced tree rows and tree management practices influence spatio-temporal dynamics of soil nutrients. Our two-year study (2021 and 2022) assessed N-leaching and soil nutrient supply at increasing distances from tree rows (0, 4, 12, 20 m); the 10-year-old TBI system (50 trees ha<sup>-1</sup>) together with agricultural controls was established in southern Québec (Canada). The TBI included hybrid poplars (*Populus deltoides* × *P. nigra*) planted alternately with high-value hardwoods in the rows. In each experimental block (n = 3), the TBI system and control were divided into two treatments: without root-pruning vs with (75 cm depth using a sub-soiler). In 2022, NO<sub>3</sub><sup>-</sup> supply rates near tree rows (0 and 4 m) were lower than alley centres (12 and 20 m) and controls. A first structural equation modelling (SEM) analysis revealed that NO<sub>3</sub><sup>-</sup> supply rates were mostly modulated by indirect effects of tree row distance and soil clay content through volumetric water content (VWC). NO<sub>3</sub><sup>-</sup> leaching (40-cm depth) at 0 and 4 m from the tree row was respectively 8.8x and 7.5x lower than that in the control. A second SEM analysis showed direct and indirect (through soil VWC affecting NO<sub>3</sub><sup>-</sup> supply rates) effects of distance from tree rows on NO<sub>3</sub><sup>-</sup> leaching rates. Within TBI greater tree leaf litter dry-mass was trapped at 0 and 4 m vs 12 and 20 m. P and K availability under tree rows was higher than all other distances within cultivated alleys and control plots. P, K, Ca and Mg supplies within cultivated alleys were generally similar among distances (4, 12, and 20 m) and did not differ from controls. An unexpected lack of effect of tree root pruning was observed regarding soil nutrient supply and N leaching. Clay content was a major driver of soil nutrient supply and N leaching. The role of TBI systems in determining soil nutrient dynamics depended upon the soil nutrient and sampling period that was measured, with greater effects beneath the trees and at the tree-crop interface.

## Introduction

Temperate tree-based intercropping systems (TBI, also denoted alley cropping systems or silvoarable systems) involve planting species that yield high quality timber or enhanced biomass production in widely spaced rows so that various agricultural crops can be grown within the intervening alleyways. These agroforestry systems have been shown to offer a wide range of ecosystem services compared to conventional agricultural systems. These services include enhancing soil nutrient cycling and water quality, increasing carbon storage, improving biodiversity and maintaining crop yield stability under climate change (Kletty et al. 2023; Mayer et al. 2022; Reyes et al. 2021; Torralba et al. 2016). Yet, ecosystem services that are offered by temperate TBI systems appear to be strongly dependent upon the edaphic and climatic context of the study and to vary widely with TBI design, management practices and geographic location. In many temperate regions where widespread implementation of TBI remains limited, greater actual assessments of multiple ecosystem services that are provided by TBI systems are needed to increase their adoption by farmers (Thiesmeier and Zander 2023).

Most early studies of temperate TBI systems generally were conducted by experimenting with relatively narrow spacings between tree rows, typically 8–20 m (Dupraz et al. 2018; Garrett et al. 2022; Thevathasan et al. 2012). Some results from these “first generation” TBI experiments suggest that deep and extensive tree roots can play an important role as a “nutrient pump,” since they can take up nutrients that are not accessible to crops, and which are then relocated to the soil surface in the alleyways as root turnover and litterfall (Isaac and Borden 2019). In fact, when they reach an advanced stage of development, trees can generate considerable amounts of litter in the form of leaves, fine roots, and rhizodeposits that increase soil organic matter, microbial diversity and activity, and nutrient

availability (Guillot et al. 2021; Rolo et al. 2023). Other studies from first-generation TBI systems also reported that deep rooting of trees can act as “safety-nets” by intercepting leached soil nutrients, especially nitrates (Allen et al. 2004a; Bergeron et al. 2011; Dougherty et al. 2009). This safety net role of trees can help mitigate groundwater and stream pollution. Despite these environmental benefits, first-generation TBI systems were shown not to be optimal from the perspective of agricultural productivity, given that tree-crop competition may rapidly arise, thereby hindering their adoption by farmers (Graves et al. 2017; Ivezić et al. 2021). Recently, second-generation temperate TBI systems, which were designed with wider spacing between tree rows (*e.g.*, 30–50 m), have produced satisfactory agricultural yields, generally equivalent to those measured in open control fields, while improving multiple soil-based ecosystem services (Carrier et al. 2019; Kanzler et al. 2019; Pardon et al. 2018; Veldkamp et al. 2023). To improve designs of second-generation TBI systems and their subsequent wide-scale adoption, there is a need to investigate the extent to which tree rows and tree management practices influence spatial and temporal dynamics of soil nutrients (especially N) within the alleyways (Cardinael et al. 2020; Kim and Issac 2022). In this study, we focus upon tree root pruning as a management practice, which was conceived to reduce nutrient and water competition between crops and trees without seriously affecting growth of the latter (Garrett et al. 2022).

The first objective of this study assessed N-leaching and soil nutrient supply at increasing distances from the tree row (0, 4, 12, 20 m) within a 10-year-old TBI system (50 trees ha<sup>-1</sup>) and in agricultural controls, *i.e.*, fields without trees and managed in the same way as the cultivated alleys in the TBI system. We hypothesized that spatial gradients of these soil properties would occur within the TBI system, with the highest nutrient supply and lowest N-leaching near the tree rows (0 and 4 m), where tree root density, litterfall and soil organic matter accumulation are greatest (Bouttier et al. 2014; Cardinael et al. 2017; Thevathasan and Gordon 1997). We expected decreasing values of nutrient supply and increasing N-leaching rates with increasing distance from the tree row. The second objective of this study determined the effect of tree root-pruning (*i.e.*, 2.5 m from the tree row) on N-leaching and soil nutrient supply. We hypothesized that tree root-pruning would increase soil nutrient availability, especially at the tree-crop interface, due to increased soil moisture (Marschner and Rengel 2023; Wanvestraut et al. 2004). We further posited that N-leaching in alleyways within the TBI system would be higher with than without tree root pruning.

## Materials and methods

### Site and field operations

The experimental site was located near Baie-du-Febvre (46°08'N, 72°43'W; *ca.* 30 m above sea level), in southwestern Québec. The 30-year climate normals (1981–2010) for the area are 5.3 °C for annual average temperature and 924 mm for annual precipitation (Nicolet meteorological station, 8 km from the site; Environment and Climate Change Canada 2023). The flat terrain has moderately good drainage. The soil is classified as a Gleysol (Soil series: Rideau clay with surface sandy patches; Laplante and Choinière 1954; Soil Classification Working Group 1998), with loamy sand texture (75% sand, 8% clay), 4.5% organic matter, and bulk pH<sub>water</sub> of 5.9 in the uppermost 0–20 cm.

The experimental site was established within an 11 ha field (560 x 200 m) with subsurface drainage, where a TBI system had been established in 2012. The TBI system consisted of three single rows of trees (northwest-southeast orientation) that were each 400 m long. The rows were composed of randomly distributed hardwoods showing similar moderate growth among species (sugar maple, *Acer saccharum* Marsh.; black walnut, *Juglans nigra* L.; northern red oak, *Quercus rubra* L.; swamp white oak, *Quercus bicolor* Willd.; bur oak, *Quercus macrocarpa* Michx.; shagbark hickory, *Carya ovata* [Mill.] K.Koch). High-value hardwoods alternated with plantings of fast-growing

hybrid poplar (*Populus deltoides* W. Bartram ex Marshall x *Populus nigra* L.). Trees were installed at 5-m spacing within the row and 40 m spacing between rows, corresponding to a density of 50 trees ha<sup>-1</sup>. In September 2021 (*i.e.*, 10 y after tree planting), average height and diameter at breast height (DBH, 130 cm) of poplars were respectively 16.1 m and 33.4 cm, while those of hardwoods were 6.4 m and 9.7 cm. Since planting, poplars and hardwoods have been maintained by annual shape-pruning (*e.g.*, removal of forks to maintain one single straight stem) and branch pruning (to less than 1/3 of the tree height). Uncultivated strips (*ca.* 2 metres-wide) were maintained under the tree rows and left unmown. “Grass strips” consisted mainly of alfalfa (*Medicago sativa* L.), Canada goldenrod (*Solidago canadensis* L.), common milkweed (*Asclepias syriaca* L.) and greater burdock (*Arctium lappa* L.). A mixture of alfalfa, timothy (*Phleum pratense* L.), tall fescue (*Festuca arundinacea* Schreb.) and meadow brome (*Bromus biebersteinii* Roem. & Schult.) was seeded under no-till on 2 May 2021. Seeding rates were 13, 8, 4 and 4 kg ha<sup>-1</sup> for alfalfa, timothy, tall fescue and meadow brome, respectively. The soil was fertilized in autumn 2020 and 2021 with 36 Mg ha<sup>-1</sup> of solid dairy cattle manure, which provided for each application *ca.* 45 kg ha<sup>-1</sup> of N, 30 kg ha<sup>-1</sup> of K and 40 kg ha<sup>-1</sup> of P. Forages were harvested three times in 2021 (18 June, 3 August, 25 October) and in 2022 (20 June, 22 July, 27 August) at a 7-cm height. Previous crops were maize (*Zea mays* L., 2017), soybean (*Glycine max* [L.] Merr., 2018 and 2019) and winter rye (*Secale cereale* L., 2020).

## Experimental design

The experimental design was divided into three replicated blocks. In each block, the TBI system was compared to an adjacent agricultural control (without trees) that included the same forage crop with the same agricultural history and management (Fig. 1). The three randomized and replicated TBI system and control plots were divided into two root-pruning treatments: without pruning *versus* with pruning. Root pruning was conducted at the end of April 2021 and 2022, which corresponded to the beginning of the growing season, using a sub-soiler (75 cm depth). In the TBI system, root pruning was implemented 2.5 m from the tree row on both sides, over a length of 40 m (Fig. 1). In each root pruning treatment, there was one transect on each side of the row and five distances from the tree row were compared: 0 m (*i.e.*, under the tree row), 4 m, 12 m, 20 m (*i.e.*, alley centre) and agricultural control (*i.e.*, > 40 m from the nearest tree) (Fig. 1).

## Measured variables

In 2021, we measured soil nitrate and ammonium bioavailability under field conditions using 2.5 cm x 10 cm anion and cation exchange membranes (Durán et al., 2013; AR204SZRA and CR67-HMR, Durpro, Candiac, QC, Canada). Within each distance plot, three pairs of membranes (one of each type) were incubated (0–10 cm depth) during two burial periods, from 10 June to 28 July (48 d) and from 9 September to 22 October (43 d), and bulked into one composite sample for both membrane types (Fig. 1; 3 blocks x 2 pruning treatments x 2 transects x 5 distances = 60 samples per burial period). After removal from the field, composite samples of membranes were rinsed with distilled water and extracted with 35 mL 2 M KCl per recovered membrane (shaking 1 h at 60 rpm), filtering the extracts (Whatman No. 42), and analyzing filtrates colorimetrically for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> (SEAL AA3 AutoAnalyzer, Folio Instruments, Kitchener, ON, Canada). Membranes were scanned to estimate contact area for each type.

Similarly, bioavailability of soil nutrients (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, P, K, Ca, Mg) was measured in 2022 using Plant Root Simulator (PRS®) probes (Hangs et al., 2004). This technology consists of cation and anion exchange membranes (adsorbing surface area of 17.5 cm<sup>2</sup>/probe) that are encapsulated in thin plastic probes. In each distance plot, four anion and four cation PRS probes were buried to a depth of 5–10 cm (2 m between pairs) during two burial periods, from 27 June to 15 July (18 d) and from 29 July to 17 August (19 d). These were bulked into one composite sample per distance for both PRS probe types (Fig. 1). Cleaned PRS probes were stored at 4°C before being sent to Western

Ag Innovations (Saskatoon, SK, Canada) for their complete analysis.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the eluant was determined colorimetrically using automated flow injection analysis (Skalar San ++ Analyzer, Skalar Inc., Breda, The Netherlands). The remaining nutrients (P, K, Ca, Mg) were measured using inductively coupled plasma spectrometry (Optima ICP-OES 8300, PerkinElmer Inc., Waltham, MA, USA).

Leaching of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  was determined using ion exchange resin lysimeters (Shelton et al. 2018; Susfalk and Johnson 2002). These passive lysimeters were constructed using PVC pipe sections of 10-cm length and diameter that contained a mixture of cation and anion exchange resins (50 g of C100E and 50 g of A400 [fresh masses], Durpro). Layers from bottom to top were: 1) 125 mL of sand (to avoid contamination from below) on top of a permeable membrane (polyester with 0.3-mm pores); 2) the resin mixture contained between two permeable membranes; 3) 150 mL of sand; 4) 150 mL of a 50:50 mixture of sand and native soil; and 5) native soil filling the remaining space. Lysimeters were deployed during two burial periods, from 28 June 2021 (1 week for deployment) to 5 May 2022 (2 weeks for retrieval and deployment) and from 5 May 2022 to 2 November 2022 (2 days for retrieval). In each plot of the downwind transects in two of the three blocks (*i.e.*, 20 plots *cf.* 60), two lysimeters were installed at 40-cm depth (top of lysimeter) under an undisturbed soil profile, ensuring good contact with the soil (Fig. 1; 2 blocks x 2 pruning treatments x 1 transect x 5 distances x 2 lysimeters / distance = 40 samples per burial period). To do so, a square pit (side  $\approx$  70 cm) with a depth of 50 cm was dug in each plot and lysimeters were put at the end of two horizontal tunnels (width and height = 10 cm; length = 40 cm), which started at the corners of the pit bottom (60 cm between tunnel entries). Tunnels were oriented at an angle so as to place the lysimeters as far as possible from the disturbed pit surface ( $\sim$  1 m between the two lysimeters). The pits were refilled after deployment. When resin lysimeters were retrieved,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were extracted from the whole resin with 500 mL 2 M KCl (shaking 1 h at 60 rpm), filtering the extracts (Whatman No. 42), and analyzing the filtrates using the SEAL AA3 AutoAnalyzer. A test of the lysimeter design was carried out using four test pits (two pits with fertilization) and confirmed that this method can capture part of the variability in  $\text{NO}_3^-$  leaching (Supplementary material S1).

Soil volumetric water content (VWC) was monitored to 7.5 cm depth on seven dates using a FieldScout TDR 350 Soil Moisture Meter (Spectrum Technologies, Aurora, IL, USA); samples taken 27 June, 27 July, 16 and 30 September in 2021, and 10 July, 30 July and 15 August in 2022. Each measurement consisted of the mean of 10 readings taken in the same 60 plots that were described previously for the ion exchange membranes, *i.e.*, 60 measurements per date. In each of the 60 plots, 6 soil cores (7 cm dia., 0–20 cm depth) were collected in July 2022. Soil cores from each plot were bulked into one composite sample. Soil texture of each sample was characterized by hydrometry (Bouyoucos, 1962).

Tree litterfall production in the TBI system was measured using litter traps (0.25 m<sup>2</sup>, 50 cm × 50 cm, 1 m above the soil surface) that were placed in every plot (except controls) of two blocks in 2021 (32 plots) and one block in 2022 (16 plots). Litter traps were deployed from 21 September to 29 November 2021, and 28 September to 24 November 2022. Litterfall (mixed, from all tree species) samples were dried (60°C to constant mass) and weighed to obtain litterfall dry mass (g m<sup>-2</sup>).

## Statistical analyses

Nutrient supply rates were calculated in terms of mass (μg) per recovered probe contact area (cm<sup>2</sup>; including both sides of the ion exchange membranes) per day. It is important to mention that the division of the raw data by the number of days does not allow the comparison between burial periods of different lengths since ion adsorption

onto membranes does not increase linearly with time (Salisbury 2000). The statistical models controlled for these differences between burial periods.

A separate model was run for each N form. The formula of the full model was:

$$N \text{ supply rate} \sim \text{Distance} \times (\text{Burial period} + \text{Root pruning}) + \% \text{Clay} + \text{random}(\text{Block/Plot}) [1]$$

where *Distance* is a categorical variable for each distance and the control, which forms an interaction with probe burial period and the root-pruning treatment. Clay percentage was used as a control for soil texture and was log-transformed to alleviate heteroscedasticity. The nested random effects control for block correlation and for repeated measurements using the same plot. Significance of each variable was evaluated with ANOVA using Type III sums-of-squares. When Distance or its interaction with the burial period was significant, multiple comparisons were automatically made (Tukey's HSD test) among distances and the control for each burial period separately. Supply rates were log-transformed prior to modelling to achieve normal distributions.

The same model and tests were used for soil VWC measurements made during the different periods of probe burial. The average of two measurement dates was used for three of four burial periods. To achieve homoscedasticity among distance classes, soil VWC was square-root-transformed.

In the case where distance from the tree row affected the supply rate of any N form, a piecewise structural equation model (SEM) was planned to allow comparisons between the direct effects of trees and their indirect effects through VWC. Since we were mainly interested in this comparison, we only use the significant fixed effects in the models for N-supply rate and VWC to construct the *a priori* SEM. Random effects were included, as shown in Eq. 1. A multi-group analysis was performed to account for interactions with burial period.

Nitrogen leaching was calculated in terms of mass (mg) per surface area (m<sup>2</sup>) per day. A separate model was constructed for each N form. The formula of the full model was:

$$N \text{ leaching} \sim \text{Distance} \times (\text{Burial period} + \text{Root pruning}) + \% \text{Clay} + \text{random}(\text{Block/Pit/Tunnel}) [2]$$

where fixed effects are the same as above (Eq. 1), except for burial period, which corresponds to lysimeter burial periods. The nested random effects control for block correlation and for repeated measurements using the same pit (one per plot) and tunnel. NO<sub>3</sub><sup>-</sup> leaching data were log-transformed to achieve normality. As noted above, ANOVA was used. For both models, we made multiple comparisons for each burial period separately, as soon as distance or its interaction with burial period was declared to be significant ( $P < 0.05$ ).

In the case where the distance to the tree row affected leaching in any N form, piecewise SEM was planned to compare direct effects of trees and their indirect effects through the corresponding N- supply rate in the topmost soil layer. Since this comparison was the only objective, the *a priori* SEM was constructed using only the significant fixed effects in the SEM for supply rate, together with significant effects in the leaching model. The random effects were included, as shown in Eqs. 1 and 2.

For incubations using PRS probes during summer 2022, supply rates for P, K, Ca and Mg were also available. The effect of trees on each macronutrient was tested using the same model as N supply (Eq. 1). Supply rates were log-transformed to achieve normality. A different model was used for dry leaf litter:

$$\text{Leaf litter} \sim \text{Distance} + \text{Year} + \text{random}(\text{Block/Plot}) [3]$$

where leaf litter mass was square-root-transformed to achieve a normal distribution and random effects are equivalent to Eq. 1. Significance of variables was determined with ANOVA in these models.

All analyses were performed using *R* version 4.2.3 (R Core Team 2023) with packages *nlme* (Pinheiro et al. 2023) for mixed models, *car* (Fox and Weisberg 2019) for ANOVA, *multcomp* (Hothorn et al. 2008) for multiple comparisons, and *piecewiseSEM* (Lefcheck 2016) for SEM.

## Results

### Nitrogen supply and volumetric water content

Nitrate supply rates were affected by a significant interaction between distance from the tree row and burial period (Table 1). In 2022, NO<sub>3</sub><sup>-</sup> supply rates near the tree row (0 and 4 m) were lower than those near the alley centre (12 and 20 m) or the control (Fig. 2a). In 2021, NO<sub>3</sub><sup>-</sup> supply rates did not vary significantly among distances.

Table 1

Significance of every predictor in the models. For each model, number of replicates, marginal (fixed effects only) and conditional (random and fixed effects) *R*<sup>2</sup>, and *P*-values are shown. The non-significant interaction "*Distance:Burial period*" had to be removed in the model for NO<sub>3</sub><sup>-</sup> leaching in order to detect a significant effect of *Distance*.

| Dependent variable in the model   |                                     |                                     |                   |                                       |                                       |                   |                   |                   |                   |
|-----------------------------------|-------------------------------------|-------------------------------------|-------------------|---------------------------------------|---------------------------------------|-------------------|-------------------|-------------------|-------------------|
|                                   | NO <sub>3</sub> <sup>-</sup> supply | NH <sub>4</sub> <sup>+</sup> supply | VWC               | NO <sub>3</sub> <sup>-</sup> leaching | NH <sub>4</sub> <sup>+</sup> leaching | P                 | K                 | Ca                | Mg                |
| <i>N</i>                          | 240                                 | 240                                 | 240               | 80                                    | 80                                    | 120               | 120               | 120               | 120               |
| Marginal <i>R</i> <sup>2</sup>    | 0.40                                | 0.62                                | 0.81              | 0.56                                  | 0.42                                  | 0.55              | 0.43              | 0.47              | 0.48              |
| Conditional <i>R</i> <sup>2</sup> | 0.58                                | 0.63                                | 0.90              | 0.78                                  | 0.69                                  | 0.81              | 0.64              | 0.78              | 0.73              |
| Independent variable (P)          |                                     |                                     |                   |                                       |                                       |                   |                   |                   |                   |
| <i>Distance</i>                   | <b>0.01</b>                         | 0.46                                | <b>&lt; 0.001</b> | <b>&lt; 0.01</b>                      | 0.91                                  | <b>0.02</b>       | <b>0.02</b>       | 0.07              | 0.72              |
| <i>Root pruning</i>               | 0.13                                | <b>&lt; 0.01</b>                    | 0.80              | 0.51                                  | 0.67                                  | 0.07              | 0.37              | 0.51              | 0.61              |
| <i>Burial period</i>              | <b>&lt; 0.001</b>                   | <b>&lt; 0.001</b>                   | <b>&lt; 0.001</b> | <b>&lt; 0.001</b>                     | <b>&lt; 0.001</b>                     | <b>&lt; 0.001</b> | <b>&lt; 0.001</b> | <b>&lt; 0.001</b> | <b>&lt; 0.001</b> |
| % <i>Clay</i>                     | <b>0.01</b>                         | <b>&lt; 0.001</b>                   | <b>&lt; 0.001</b> | 0.91                                  | 0.65                                  | <b>&lt; 0.001</b> | <b>0.03</b>       | <b>&lt; 0.001</b> | <b>&lt; 0.001</b> |
| <i>Distance : Root pruning</i>    | 0.24                                | 0.12                                | 0.15              | 0.97                                  | 0.50                                  | 0.34              | 0.63              | 0.71              | 0.90              |
| <i>Distance : Burial period</i>   | <b>&lt; 0.001</b>                   | <b>0.04</b>                         | <b>&lt; 0.001</b> | n/a                                   | <b>0.04</b>                           | <b>&lt; 0.01</b>  | 0.09              | <b>&lt; 0.001</b> | <b>&lt; 0.001</b> |

$\text{NO}_3^-$  supply rates were about 11 times higher than those of  $\text{NH}_4^+$ . There was significant interaction between distance and burial period that affected  $\text{NH}_4^+$  supply rates (Table 1). Overall,  $\text{NH}_4^+$  supply rates varied weakly among distances, except during the second burial period in 2022:  $\text{NH}_4^+$  supply at 20 m was greater than at 0 m (Fig. 2b).  $\text{NH}_4^+$  supply rates (mean  $\pm$  SE) in plots with root pruning ( $5.0 \pm 0.5 \times 10^{-2} \pm 0.0051 \mu\text{g cm}^{-2} \text{d}^{-1}$ ) were significantly (Table 1), but slightly higher than plots without root pruning ( $4.3 \pm 0.4 \times 10^{-2} \mu\text{g cm}^{-2} \text{d}^{-1}$ ).

Significant interactions between distance and sampling period characterized soil volumetric water content (VWC) (Table 1). On all sampling dates, VWC at 0 m was lower than that at other distances from the tree row (Fig. 2c). In 2022, VWC was highest near the alley centre (12 and 20 m).

We tested direct and indirect effects of distance from the tree row and clay content on  $\text{NO}_3^-$  supply rates with an SEM (Fig. 3). It showed how distance affected  $\text{NO}_3^-$  supply rates only through soil VWC, since the direct effect on  $\text{NO}_3^-$  was not significant. The effect of clay content on  $\text{NO}_3^-$  supply rates was also mainly mediated by soil VWC.

## Nitrogen leaching

Nitrate leaching varied significantly among distances from the tree row (Table 1). Regardless of burial period of the lysimeters, rates of  $\text{NO}_3^-$  leaching near the tree row (0 and 4 m) were lower than near the alley centre (12 and 20 m) and the control (Fig. 4). To detect the significant effect of distance, the interaction between distance and the burial period had to be removed. Nevertheless, we examined differences among distances and control for each burial period separately, since they represent different seasons between which leaching dynamics could change substantially. In both burial periods, lysimeters at 0 and 4 m intercepted less  $\text{NO}_3^-$  compared to those at 12 m, 20 m or the control ( $P < 0.05$ ).

Rates of  $\text{NO}_3^-$  leaching were about 12 times lower than rates of  $\text{NH}_4^+$  leaching (results not shown). A significant interaction between distance and burial period affected  $\text{NH}_4^+$  leaching rates (Table 1). In examining differences among distances and the control for each year separately, no effect was found for  $\text{NH}_4^+$  leaching rates (results not shown).

We tested direct and indirect effects of distance from the tree row, clay content and period of lysimeter burial on  $\text{NO}_3^-$  leaching rates with an SEM (Fig. 5). The model showed how distance from the tree row affected indirectly  $\text{NO}_3^-$  leaching through soil VWC affecting  $\text{NO}_3^-$  supply rates in the uppermost soil layer. Yet, the SEM also detected a strong direct effect of distance on  $\text{NO}_3^-$  leaching rates, which was not mediated by  $\text{NO}_3^-$  supply rates.

## Leaf litter accumulation and supply of P, K, Ca and Mg

Within the TBI system, greater leaf litter dry mass was trapped at 0 and 4 m compared to 12 and 20 m (Fig. 6). The effect of distance or the interaction between distance and burial period was significant for P, K, Ca and Mg supplies (Table 1). Supplies of P and K beneath the tree row (0 m) were higher than within the cultivated alleys (4, 12 and 20 m) and in the control (Fig. 7). Supply of P, K, Ca and Mg within the cultivated alleys was generally similar across distances and did not differ from that in the control.

## Discussion

**Supply and leaching of nitrates decreased near the tree row independently of tree root pruning treatments**

Contrary to our prediction,  $\text{NO}_3^-$  supply rates near the tree row (0 and 4 m) were significantly lower than those near the alley centre (12 and 20 m) and in the agricultural control. Yet, high amounts of tree litter accumulated at 0 and 4 m (Fig. 6), which likely led to substantial increases in soil organic matter content and total N (Pardon et al. 2017). Our first SEM analysis revealed that decreased  $\text{NO}_3^-$  supply rates in the vicinity of the tree row were mainly driven by a soil moisture (VWC) reduction (Fig. 3). Other studies found positive relationships between soil moisture and  $\text{NO}_3^-$  supply that was measured with ion exchange membranes (Hofer et al. 2017; Rolo et al. 2012). Moisture limitation in soils generally induces inhibition of enzymatic activity and a decrease in microbial activity, thereby resulting in declines in soil nitrification (Gao et al. 2020). As tree root pruning was applied at 2.5 m distance from each side of the tree, it is not surprising that soil VWC and  $\text{NO}_3^-$  supply under the tree row (0 m) were unaffected by this tree management practice. Aboveground conditions were likely similar between both treatments of tree-root pruning in this uncultivated strip. Previous studies in our experimental site found a weak effect of tree root pruning on tree growth (Gagné et al. 2022). Accordingly, tree root pruning likely had a negligible indirect effect on tree aboveground traits (*e.g.*, canopy width, total leaf area) that can influence soil VWC through light and water interception by trees. Strong reduction in soil moisture under the tree row (Fig. 2c) could be due to water interception and uptake by trees and volunteer herbaceous species that were not mowed (Jacobs et al. 2022).

Soil VWC (one date) and  $\text{NO}_3^-$  supply rates (two dates) at 4 m from the tree row were substantially reduced compared to that in the control, in both tree root-pruning treatments (Table 1, Fig. 2a, Fig. 2c). This lack of effect of tree root pruning at the tree-crop interface (4 m) was unexpected. Increases in soil moisture at the tree-crop interface due to the physical separation of crop and tree roots were observed in previous TBI experiments (Miller and Pallardy 2001; Wanvestraut et al. 2004). It is possible that net plant uptake (trees included) of water and  $\text{NO}_3^-$  was comparable in both treatments. Yet, two different scenarios can explain this response: 1) lower demand by forage plants (compensated by tree demand) as a result of intense tree root competition in the soil surface of the plots without root pruning, and 2) higher consumption of these resources by the forage crop in the plots with root pruning as a result of negligible tree root competition (Allen et al. 2004b; Hou et al. 2003). The conjecture of the first scenario is supported by data from Bouttier et al. (2014). The authors observed substantial root density and overlap between trees (hybrid poplar and red oak) and a forage crop in the soil surface in an 8-year-old TBI system that was established in southern Quebec, under edapho-climatic conditions that were similar to those in the present study. They further observed that 40% (trees) to 50% (forage crop) of the fine roots were located in the uppermost 10 cm of soil. The conjecture for the second scenario is supported by data from Hébert et al. (unpublished results), who performed a companion study on the site of the present study. They found that overall aboveground dry-mass of the forage crop (*i.e.*, mean of six cuts in 2021 and 2022) at 4 m from the tree row in the plots with tree root pruning was 20% higher than that in the plots without root pruning. It is also possible that control of tree root competition at 4 m from the tree row by root pruning was quite ephemeral as trees, especially fast-growing species such as poplar, have a high capacity for rapid root regrowth in the root exclusion zone following root pruning (Jin et al. 2017; Wajja-Musukwe et al. 2008). We believe the sub-soiler employed in the root pruning treatment did not result in a confounding effect on soil hydrology as soil VWC in the control plots did not vary between root pruning treatments throughout the study (Table 1).

Nitrate leaching at 0 and 4 m from the tree row was respectively 8.8x and 7.5x lower than that in the agricultural control regardless of burial period and the tree root-pruning treatment (Fig. 4). Our second SEM analysis indicated that decreased  $\text{NO}_3^-$  supply in the vicinity of the tree row, which was indirectly driven by a soil moisture reduction in the soil surface, contributed to decreased  $\text{NO}_3^-$  leaching (Fig. 5). Reduction in soil water availability in croplands

may limit drainage and net nitrification and, accordingly,  $\text{NO}_3^-$  leaching (Dayryanto et al. 2017; Thapa et al. 2018). Abundance of soil microorganisms carrying denitrification genes also could increase close to tree rows in TBI systems compared to alley centres or agricultural controls, suggesting a greater potential for  $\text{NO}_3^-$  removal through denitrification, which may lead to reduced  $\text{NO}_3^-$  leaching (Beule et al. 2020).  $\text{NO}_3^-$  leaching rates at 4 m from the tree row did not significantly vary between tree root pruning treatments (Table 1), contrary to expectation and to findings from other TBI studies. In a 8-year-old TBI system that was established in southern Québec, Bergeron et al. (2011) estimated that the presence of hybrid poplar roots in non-trenched plots decreased subsoil (0–70 cm depth)  $\text{NO}_3^-$  leaching at 2 m from the tree row by 227 kg N ha<sup>-1</sup> and 30 kg N ha<sup>-1</sup> over two consecutive years compared to trenched plots. At 0 m (with and without tree root pruning), tree roots likely played an important role in scavenging soil  $\text{NO}_3^-$  leachate, a process that is commonly labelled as the “safety-net role of trees” (Allen et al. 2004a). In relatively young TBI systems, tree fine root density is generally concentrated close to the tree rows (Bouttier et al. 2014; O'Connor et al. 2023). At 4 m in the TBI plots, reduction of  $\text{NO}_3^-$  leaching was likely due to unclear processes that governed soil moisture and N fluxes, which were discussed in the previous paragraph.

Overall,  $\text{NH}_4^+$  supply and leaching rates were very low compared to  $\text{NO}_3^-$ , which is consistent with other studies that have used the same analytical methods as the present study (Fortier et al. 2019; Shelton et al. 2018). Scarcity of  $\text{NH}_4^+$  in soil solution is not surprising given that it is an exchangeable ion that is easily adsorbed to soil colloids; under favourable conditions,  $\text{NH}_4^+$  can be rapidly converted to  $\text{NO}_3^-$  through nitrification (Whalen et al. 2013).

## Spatial variation in supplies of P, K, Ca and Mg

Supplies of P and K under the tree row (0 m) increased compared to those within the cultivated alleys (4, 12 and 20 m) and in the control (Fig. 7). Different mechanisms have been proposed to account for nutrient accumulation beneath tree canopies in TBI systems. These include litter accumulation (Fig. 6), movement of nutrients upward from deeper soil layers (*i.e.*, nutrient pumping by tree roots) and inputs delivered by throughfall water (Isaac and Borden 2019; Pardon et al. 2017). While litter inputs from young trees in TBI systems occur mainly at short distances from them, the positive effects of trees on nutrient supplies can extend increasingly further away from tree rows over time as trees grow larger and tree litter becomes more evenly dispersed within the alleyways (Thevathasan et al. 2018). Further field and modelling studies are required to determine more accurately the effect of TBI systems on soil nutrient supplies at different stages of tree development. The lack a tree root-pruning effect on soil supplies of P, K, Ca and Mg (Table 1) and their relatively even distribution among different distances within the cultivated alleys (Fig. 7) suggests that tree-crop competition for these nutrients was likely negligible.

## Clay content as key driver of soil nutrient dynamics in TBI experiments

Clay content played a significant role in determining variation in soil VWC, nutrient supply and leaching of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Table 1, Fig. 3, Fig. 5). In our experimental plots, clay content varied from 3.5 to 17.6%, with mean coefficients of variation (CV) of 35% and 43% within and between blocks, respectively. TBI experiments are generally established on relatively large agricultural areas compared to most agronomic experiments, which may induce substantial soil textural heterogeneity. As pointed out by others, we assert the importance of accounting for heterogeneity of abiotic factors in statistical modelling, such as soil texture, which is often an intrinsic feature of field experiments in agroforestry (Beuschnel et al. 2019).

# Conclusion

This study assessed the effect of tree root pruning on N-leaching and soil nutrient supply at different distances from the tree row within a 10-year-old TBI system. Significant decreases in rates of  $\text{NO}_3^-$  supply and leaching were measured close to the tree row independent of whether tree roots were pruned or not. The decrease in  $\text{NO}_3^-$  availability in the soil surface through moisture reduction was an important determinant of  $\text{NO}_3^-$  leaching attenuation near the tree row. Supplies of P, K, Ca and Mg weakly varied within the cultivated alleys and were unaffected by tree root pruning, thereby suggesting negligible tree-crop competition for these macronutrients. Effects of TBI on soil nutrient dynamics varied widely according to the soil nutrient being measured, the sampling period, and the inherent soil textural heterogeneity within the experimental site, with greater responses occurring beneath the tree row and at the tree-crop interface.

# Declarations

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## Author contributions

All authors contributed equally to the study conception and design. Material preparation, data collection and analysis were performed by Marc-Olivier Martin-Guay. The first draft of the manuscript was written by David Rivest. All authors read and approved the final manuscript.

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## Competing interests

The authors declare no competing interests.

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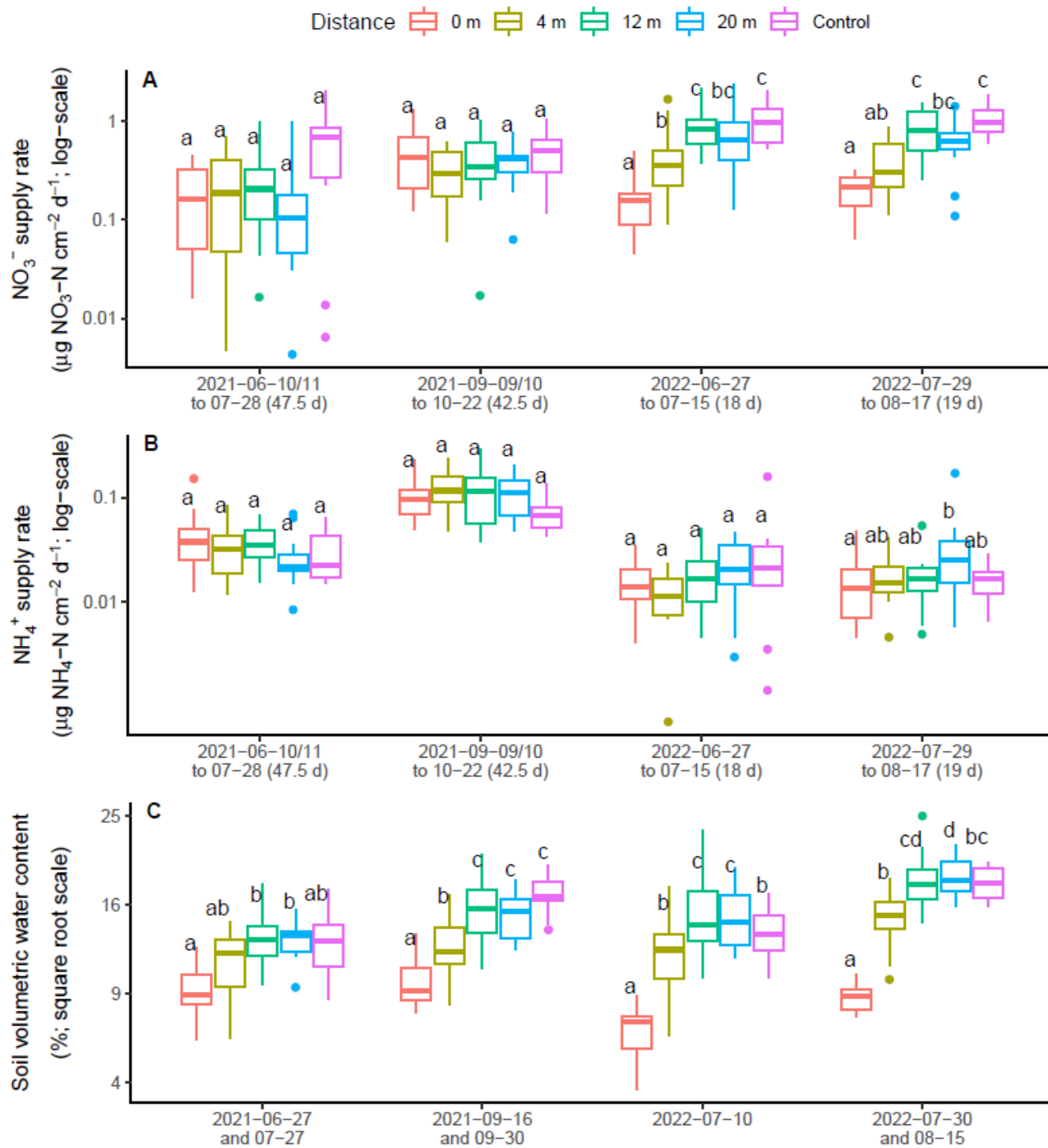
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## Figures



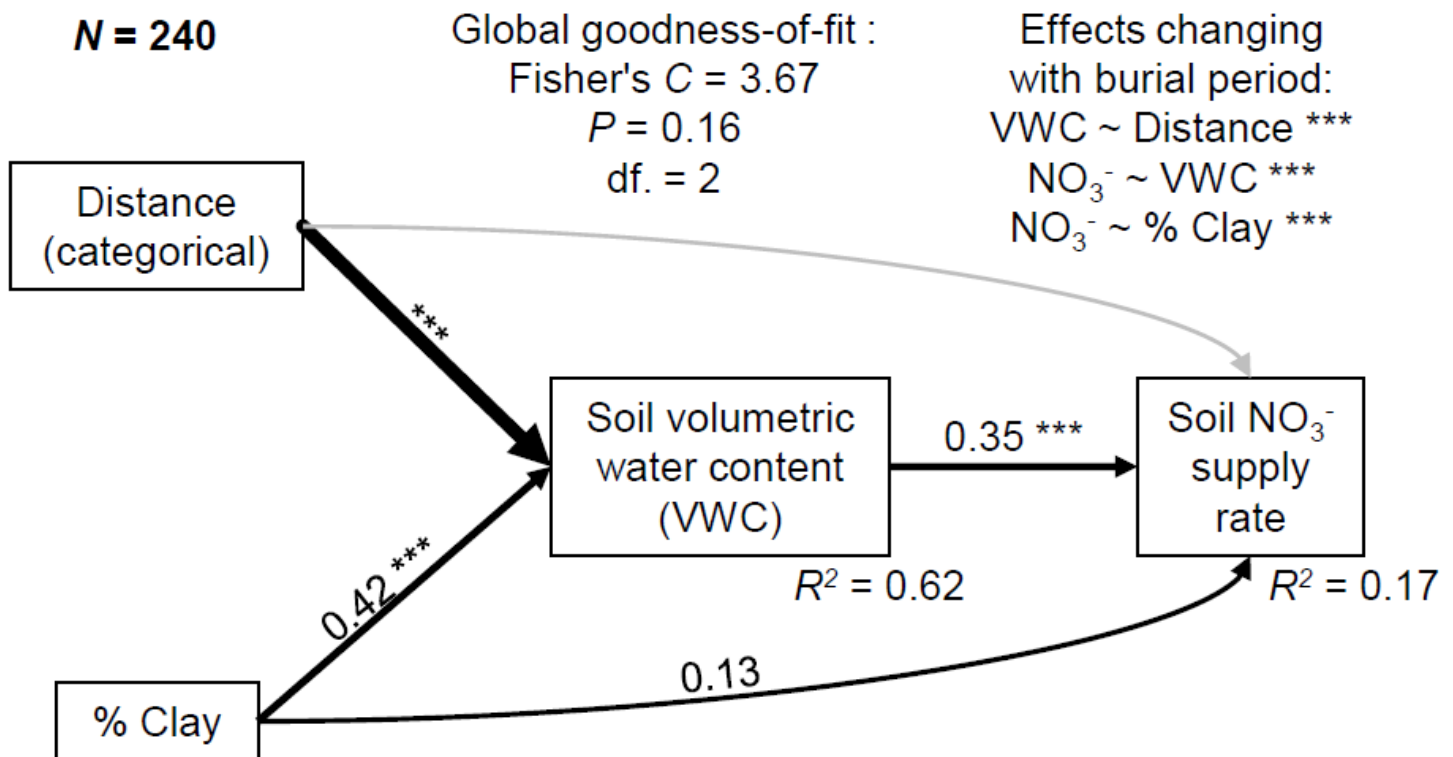
**Figure 1**

Block plan (not to scale) of the experimental design that was implemented in 2021 and 2022 in the tree-based intercropping system. Blocks were replicated three times. Soil was repeatedly measured at different distances from the tree row. Each plot contained three (2021) to four (2022) pairs of membranes that were located 2 m apart.



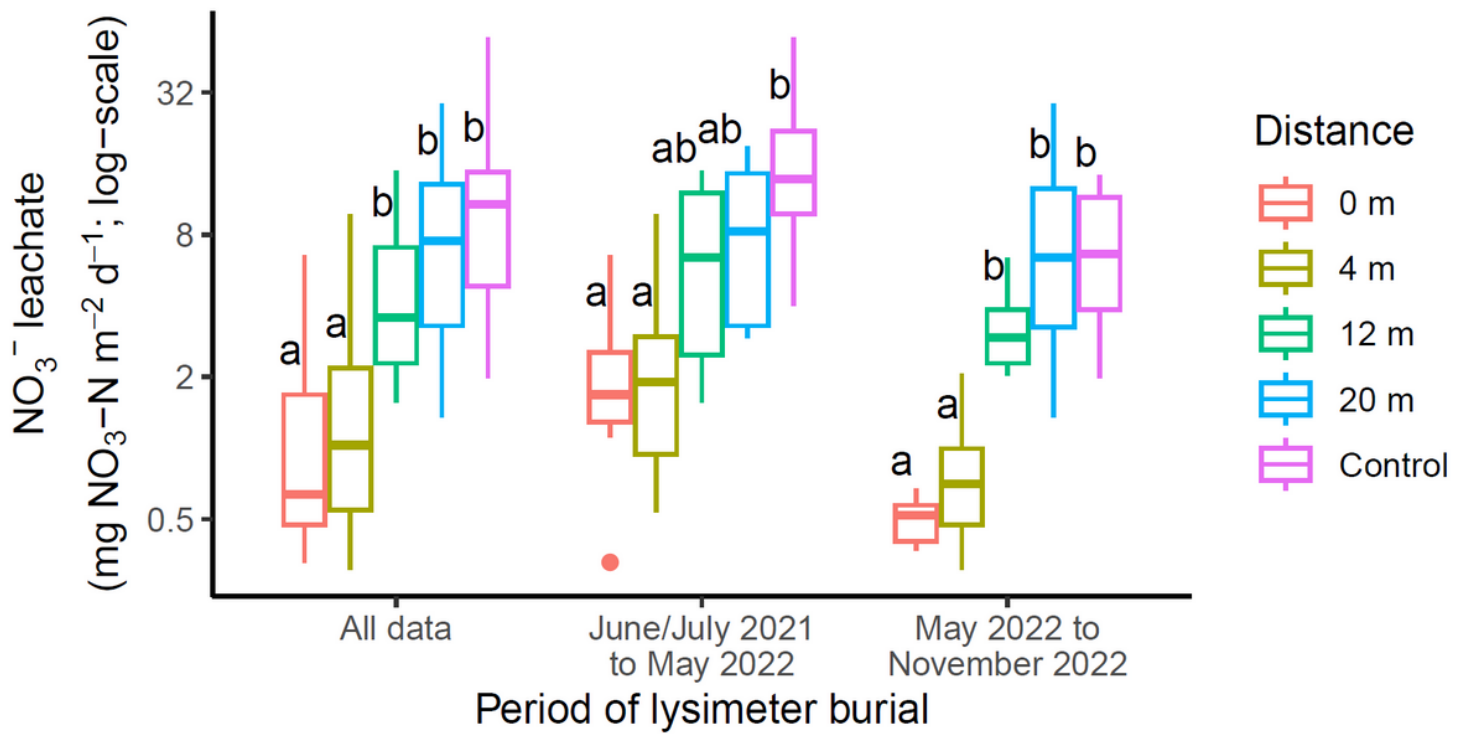
**Figure 2**

$\text{NO}_3^-$  supply rate (A),  $\text{NH}_4^+$  supply rate (B), and soil volumetric water content (C) at different distances from the tree row in the tree-based intercropping system and in the control. Within each sampling period, different letters represent a significant difference (Tukey HSD for multiple comparisons,  $P < 0.05$ ). Box-and-whisker plots display 25<sup>th</sup> and 75<sup>th</sup> percentiles (the inter-quartile range [IQR] from the lower and upper edges of the box), the bold horizontal lines within boxes indicate the 50<sup>th</sup> percentiles (medians); whiskers below and above boxes indicate farthest values not farther than  $1.5 \times \text{IQR}$  from their corresponding quartiles, beyond which dots indicate outliers.



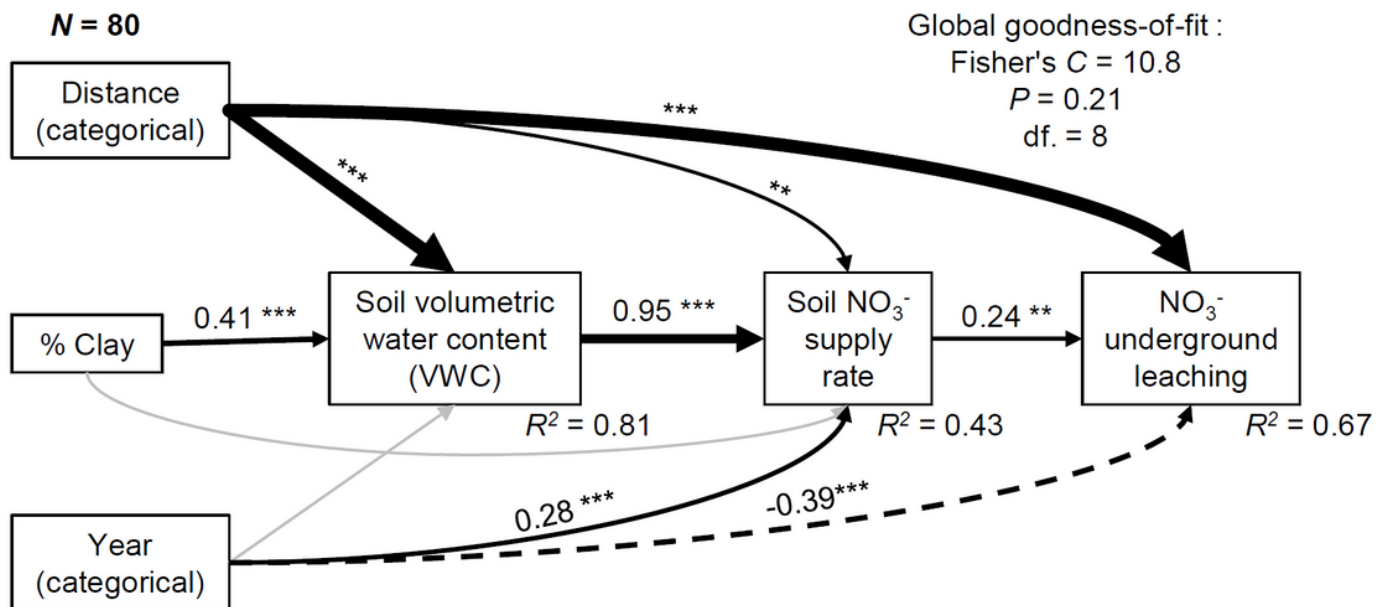
**Figure 3**

Piecewise structural equation model (SEM) to test direct and indirect effects of distance from trees (categorical variable including agricultural control) on soil  $\text{NO}_3$  supply rate. The direct effect was removed from the saturated SEM since it was not significant. A multi-group analysis was performed to account for interactions with burial period. Global goodness-of-fit shows that we cannot reject this model and that adding the missing link (grey) does not substantially improve the SEM. Arrow sizes represent the importance of each link, based upon standardized coefficients, except for distance importance, which was based upon the proportion of VWC variance explained by distance alone (*i.e.*, the reduction in marginal  $R^2$  when removing "VWC ~ Distance") compared to clay percentage alone. Marginal  $R^2$ -values are shown. \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



**Figure 4**

$\text{NO}_3^-$  leaching rate at different distances from the tree row in tree-based intercropping system and in the control. Within each period of lysimeter burial, different letters represent significant differences (Tukey HSD for multiple comparisons,  $P < 0.05$ ).



**Figure 5**

Piecewise structural equation model (SEM) to test direct and indirect effects of distance from trees (categorical variable including agricultural control) on  $\text{NO}_3^-$  underground leaching. This SEM was built upon the SEM in Fig. 3

using plots from the leaching experiment. Non-significant links were removed (grey arrows) and "NO<sub>3</sub><sup>-</sup> supply ~ Distance" was added since the test of directed separation was significant. Global goodness-of-fit shows that we cannot reject this model. Arrow sizes represent the importance of each link based upon standardized coefficients (dashed when negative), except for distance importance, which was based upon the proportion of variance explained by distance alone (*e.g.*, the reduction in marginal  $R^2$  when removing "VWC ~ Distance") compared to each of the other variables. Marginal  $R^2$ -values are shown. Burial period has coefficients, since there are only two categories. \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

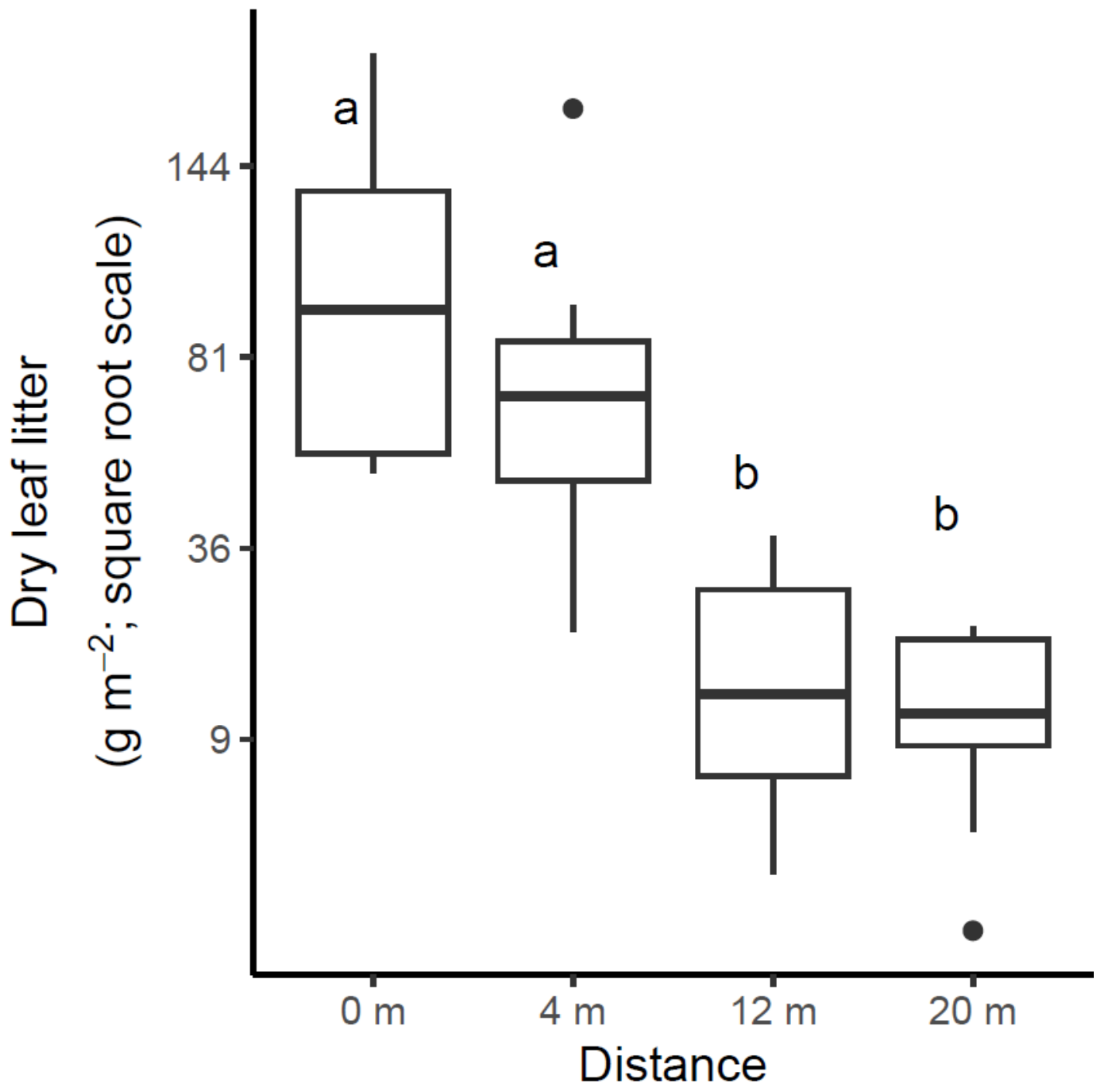
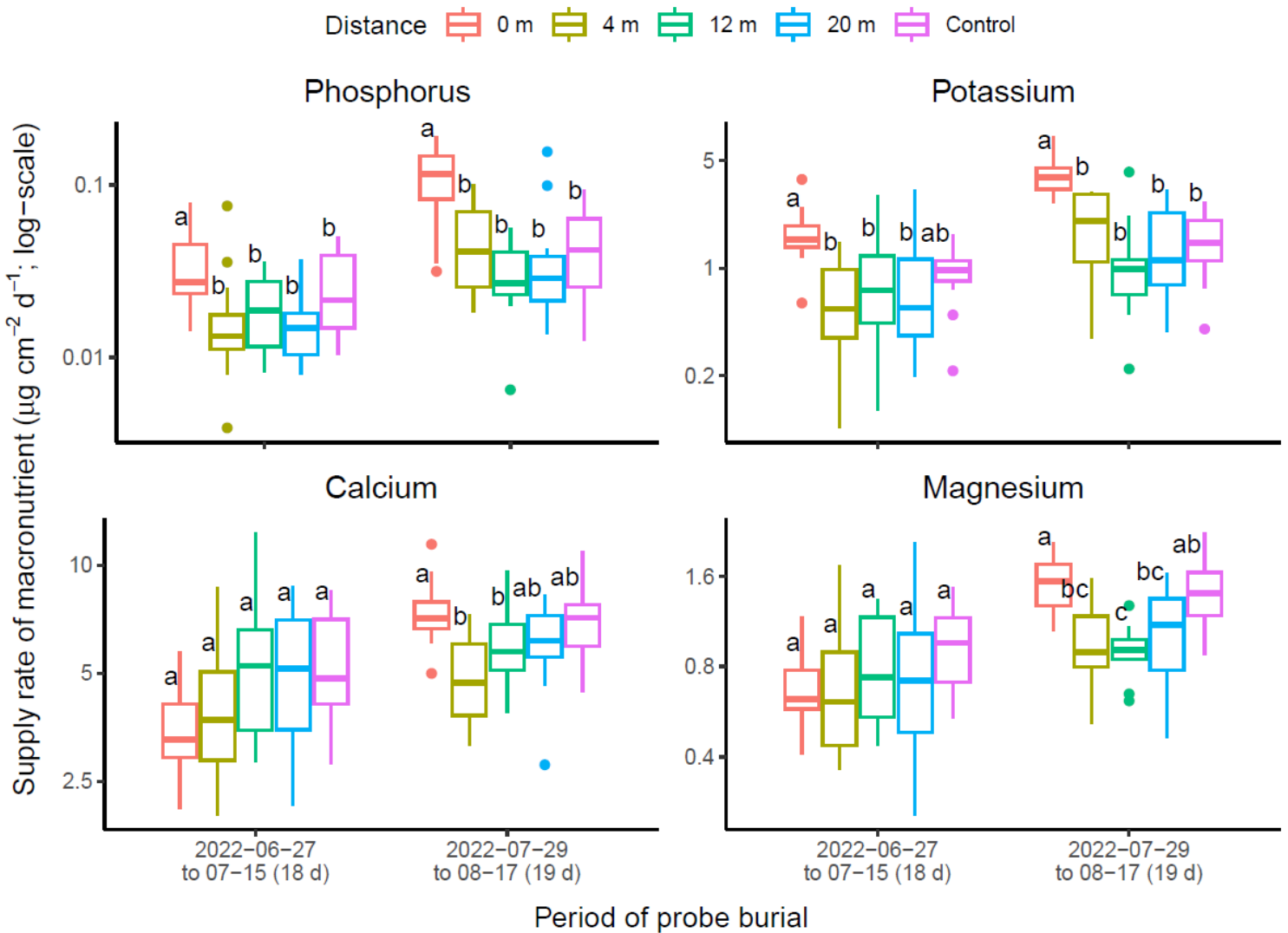


Figure 6

Dry tree leaf litter accumulation at different distances from the tree row in the tree-based intercropping system. Different letters represent significant differences (Tukey HSD for multiple comparisons,  $P < 0.05$ ).



**Figure 7**

Supply rates of Phosphorus, Potassium, Calcium and Magnesium at different distances from the tree row in tree-based intercropping system and in the control. Within each sampling period, different letters represent significant differences (Tukey HSD for multiple comparisons,  $P < 0.05$ ).

## Supplementary Files

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