Received: 30 June 2024 | Accepted: 15 May 2025

DOI: 10.1002/agj2.70098

Agronomy Journal

ORIGINAL ARTICLE

Agronomy, Soils, and Environmental Quality

Annual ribwort plantain and alfalfa mixtures enhance forage accumulation and reduce nitrate

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Assigned to Associate Editor Lisa Baxter.

Funding information
eip-agri Agriculture & Innovation—Rural development 2014-2020 for Operational Groups (in the sense of EU Art 56 of Reg. 1305/2013), Grant/Award Number: 332018001101LWC; Dresden University of Applied Sciences

Abstract

Nitrate (NO_3) leaching from alfalfa (*Medicago sativa* L.) cultivation in autumn and sustaining high forage accumulation under dry conditions is a serious problem in farming. In this study, mixtures of alfalfa and ribwort plantain (*Plantago lanceolata* L.) had agronomic advantages for forage accumulation compared to corresponding mixtures of alfalfa and a grass species (meadow fescue [*Festuca pratensis Hudson*]). The ribwort plantain and alfalfa mixtures accumulated twice as much forage as the reference mixtures with meadow fescue. Most of the forage accumulation was accounted for ribwort plantain due to poor initial alfalfa development. Ribwort plantain suppressed alfalfa and all weed species from as early as July and continued increasing forage accumulation until early autumn. At the same time, ribwort plantain contributed more than meadow fescue to a reduction in NO_3-N. Significantly lower NO_3-N shares in the soil were observed, on average, in mixtures with ribwort plantain and alfalfa in the 0.6- to 1.2-m soil depths in autumn. Due to the inhibition of nitrification by ribwort plantain, NH_4-N was present in the soil solution of the sandy soil at the trial site to a greater extent in autumn compared to alfalfa and the mixtures with meadow fescue. Due to the highly competitive power of ribwort plantain, it should only be sown in mixtures with alfalfa at seed rates of 100 germinating seeds m^{-2}.

Plain Language Summary

Nitrate (NO_3) leaching from alfalfa (*Medicago sativa* L.) cultivation in autumn and sustaining high forage accumulation under dry conditions is a serious problem in farming. In this study, mixtures of alfalfa and ribwort plantain (*Plantago lanceolata* L.) had agronomic advantages for forage accumulation compared to corresponding mixtures of alfalfa and a grass species (meadow fescue [*Festuca pratensis Hudson*]). The ribwort plantain and alfalfa mixtures accumulated twice as much forage as the reference mixtures with meadow fescue. Most of the forage accumulation

Abbreviations: LER, land equivalent ratio; pLER, partial land equivalent ratio.

was accounted for ribwort plantain due to poor initial alfalfa development. Ribwort plantain suppressed alfalfa and all weed species from as early as July and continued increasing forage accumulation until early autumn. At the same time, ribwort plantain contributed more than meadow fescue to a reduction in NO_3-N. Significantly lower NO_3-N shares in the soil were observed, on average, in mixtures with ribwort plantain and alfalfa.

1 | INTRODUCTION

The goal of organic farming is to develop environmentally sustainable and integrated crop production systems that work similarly to natural ecosystems (Rigby & Cáceres, 2001). Organic cultivation methods completely exclude the use of synthetic nitrogen (N) fertilizers (Hansen et al., 2000; Knapp et al., 2023), thus organic farming practices must provide a sufficient N supply through symbiotic N_2 fixation to maintain a moderate yield level (Barbieri et al., 2021). In organic farming, N loss in a certain crop growth cycle leads to higher yield losses compared to conventional farming practices, which can compensate N losses easier through mineral fertilizers (Pandey et al., 2018).

Due to the ability of fodder legumes to symbiotically fix N, they are a necessary component of crop rotations in organic farming (Fustec et al., 2010; Kayser et al., 2010). Alfalfa (*Medicago sativa* L.) is a high-yielding, deep-rooted, and crude protein-rich fodder legume capable of symbiotically fixing high amounts of N; it is also characterized by high drought tolerance (Ghimire et al., 2021; Moghaddam et al., 2015). Alfalfa is capable of achieving higher yields than other fodder legumes (e.g., clovers) on organic farms (Liu et al., 2022). However, alfalfa cultivation can produce very high quantities of NO_3-N that are accumulated as a result of the input of high and easily mineralizable organically bound N quantities (Heichel et al., 1984). Alfalfa can fix between 93 and 183 kg N ha^{-1}, on average, in the first year (Burity et al., 1989; Kelner et al., 1997). Rakotovololona et al. (2019) reported an average of 333 kg N ha^{-1} fixed by alfalfa in pure stands. Numerous studies have shown that NO_3-N leaching in pure alfalfa stands is comparatively high in autumn of the first year (Burity et al., 1989; Heichel et al., 1984, 1985; Masoni et al., 2015; Rakotovololona et al., 2019; Schmidtke, 2001; Ta & Faris, 1987; Walley et al., 1996). This creates a concern for organic farming as a means by which loss of critical resources to maintain yield and retain N in the system may occur. NO_3-N leaching from perennially grown alfalfa is potentially lower (Benoit et al., 2014), which is associated with a high N content in harvested biomass and a greater water depletion through the roots of alfalfa (Singh et al., 2023).

Alfalfa is often mixed with grass species, which is beneficial for farming systems (Fernandez et al., 2019). In many cases, alfalfa and grass mixtures achieve higher yields compared to pure stands (Aponte et al., 2019; Fernandez et al., 2019; Sleugh et al., 2000; Veronesi et al., 2010). Meadow fescue (*Festuca pratensis* Hudson) is a valuable grass for alfalfa mixtures because it has a rather low competitive ability due to its slower establishment (Cherney et al., 2020; Flynn et al., 2013; Suter et al., 2004). Meadow fescue has a good fodder value, comparable to English ryegrass (*Lolium perenne* L.) (Suter et al., 2011). It is mainly found in low- to medium-intensity managed natural pastures, but it is also suitable for intensively managed meadows because of its high fodder value (Frick et al., 2019). Alfalfa and meadow fescue grow well together as an annual mixture because they both have similar growth seasons, drought tolerance, and a high fodder value (Hartmann, 2013; Kivelitz, 2020; Landwirtschaftskammer NRW, 2025; LfL, 2025).

Ribwort plantain (*Plantago lanceolata* L.) is a widely distributed plant species in meadows and pastures across Central Europe (Pol et al., 2021) and is palatable for grazing animals (Cavers et al., 1980; Rumball et al., 1997; Stewart, 1996). Most frequently, ribwort plantain in agriculture is as a component of mixed pastures (Stewart, 1996). However, ribwort plantain offers agronomic properties that have received little recognition in agriculture but have potential to increase the resilience and yield of plant stands in organic farming. Ribwort plantain has an average root length density of 1.6 cm^{-3} in 0- to 0.75-m soil depth and a specific root length up to 1.9 m, which can increase the resilience of cropping systems during drought periods under climate change (Pol et al., 2021). Ribwort plantain seeds germinate well under field conditions (Sagar & Harper, 1960), are insensitive to light conditions (Ellenberg & Leuschner, 2010; Thompson & Grime, 1983) and temperature changes (Thompson & Grime, 1983), and germination temperatures range from 13.5 to 30.5˚C (Grey et al., 2019). Ribwort plantain is an encroaching species that can also be established through dormant seeding in winter (Grey et al., 2019; Pons & van der Toorn, 1988).

Furthermore, ribwort plantain contains aucubin, an iridoid glycoside that inhibits the nitrification of NH_4-N and consequently reduces the (NO_3-N) content in the soil (Dietz et al., 2013). The secondary components acteoside (phenylpropanoid) and catalpol also contribute to the inhibition of nitrification (Carlton et al., 2019). Moreover, ribwort plantain reduces nitrous oxide gas (N_2O) formation in grasslands on peat soil by 39% compared to ryegrass (Pijlman et al., 2020) and by 50% in pastures (Gardiner et al., 2018) and reduces NO_3-N leaching by 19% in grazing systems (Navarrete et al., 2022).

Previously developed agronomic strategies to reduce NO_3-N leaching after the plowing of alfalfa in autumn, such as the cultivation of catch crops or main crops with high N uptake, postponement of the plowing date, and reduction of soil cultivation intensity, reduced nitrate (NO_3) losses after the plowing of alfalfa in winter (Dreymann et al., 2005; Heß, 1989; Koch, 1997; Notaris et al., 2018) but did not lead to a sustainable solution for the problem in organic farming. As a result, a novel climate-resilient cultivation strategy for alfalfa plowing in autumn is needed to generate high yields under climate change, to reduce NO_3 leaching in winter, and to diversify crop rotations in organic farming.

Despite the putative benefits of ribwort plantain, research regarding viability and sustainability in cultivation with alfalfa has not been done. Therefore, the objective of this study was to compare pure annual stands and different mixtures of alfalfa with ribwort plantain and meadow fescue under organic conditions for the first time. We hypothesized that (a) a mixture of alfalfa and ribwort plantain would result in a faster decline in the NO_3-N content in the soil layer compared to the reference mixture with meadow fescue by increasing the proportion of ammonium nitrogen (NH_4-N); and (b) a mixture of alfalfa with ribwort plantain would be higher yielding than a mixture of alfalfa with meadow fescue under increasing water shortage during the growing season.

2 | MATERIALS AND METHODS

2.1 | Experimental site and design

Different mixtures of ribwort plantain, alfalfa, and meadow fescue and pure stands were established at the same location in Canitz, Germany (near Leipzig, Germany) during 2019 and 2021. The site is located 112 m above sea level at 51°24′45″ N 12°41′10″ E. The field trial was in a water protection area. According to the Federal Water Resources Act (Bundesministerium der Justiz und für Verbraucherschutz, 2009/22.12.2023), the Fertilizer Regulation in Germany (DüV, 2017/11.12.2024) and the European Union Nitrate Directive (European Union, 1991), the upper limit for the application of N is 170 kg ha^{-1} during specific periods on arable land in water protection areas. If the NO_3^- content in the groundwater exceeds 50 mg L^{-1}, any fertilization with liquid manure, slurry, compost, or solid manure is strictly prohibited. Previous site management and winter crop management are described in Table 1. The Canitz field has been farmed organically with this crop rotation since 1990 (Table 1).

Core Ideas

- Alfalfa in pure stands and in grass mixtures leads to nitrate losses in autumn.
- Plant-available nitrogen was reduced under alfalfa with ribwort plantain in autumn.
- Ribwort plantain shifted nitrate nitrogen to ammonium in alfalfa mixtures in autumn.
- Mixture of ribwort plantain accumulated twice as much forage as meadow fescue mixtures.
- Meadow fescue had a lower partial LER than ribwort plantain.

TABLE 1 Crop rotation for both study trials on the site Canitz for the period 2016–2021.

Crop	Year	Crop rotation
Alfalfa (*Medicago sativa* L.)	2016–2017	Main crop
Spelt (*Triticum spelta* L.)	2018	Main crop
Phacelia (*Phacelia tanacetifolia* L.)	2018–2019	Catch crop
Field pea (*Pisum sativum* L.)	2019	Main crop
Oil radish (*Raphanus sativus* L.)	2019–2020	Catch crop
Potatoes (*Solanum tuberosum* L.)	2020	Main crop
Phacelia (*Phacelia tanacetifolia* L.)	2020–2021	Catch crop

The plots were arranged in a randomized block design with four replicates and random treatment allocations in each trial year (Figure 1A). The composition of ribwort plantain, alfalfa, and meadow fescue comprised a de Witt replacement series design from 0% to 100% of the respective plant species (alfalfa, ribwort plantain, and meadow fescue) to cultivate plant species in pure stands (100%) and pair them with a mixture of species in increasing percentages, as described by deWit (1960). Therefore, the following forage species mixtures were tested: (1) 100% ribwort plantain; (2) 67% ribwort plantain and 33% alfalfa; (3) 50% ribwort plantain and 50% alfalfa; (4) 33% ribwort plantain and 67% alfalfa; (5) 100% alfalfa; (6) 67% alfalfa and 33% meadow fescue; (7) 50% alfalfa and 50% meadow fescue; (8) 33% alfalfa and 67% meadow fescue; and (9) 100% meadow fescue. A plot was comprised of four drill passes for a total width of 7.2 m in width and 10 m in length (Figure 1A). The total experimental field dimensions were 85.2 m × 55 m. The field trials were set up on April 1, 2019, and March 22, 2021.

All measurements and samplings described in the following sections took place within the second and third drill passes of the plots. The first and fourth drill passes minimized the border effects of the neighboring plots.

The 2 years of study were characterized by very different weather conditions. The warmest year was 2019 with an average temperature of 12.5°C and trial year 2021 was

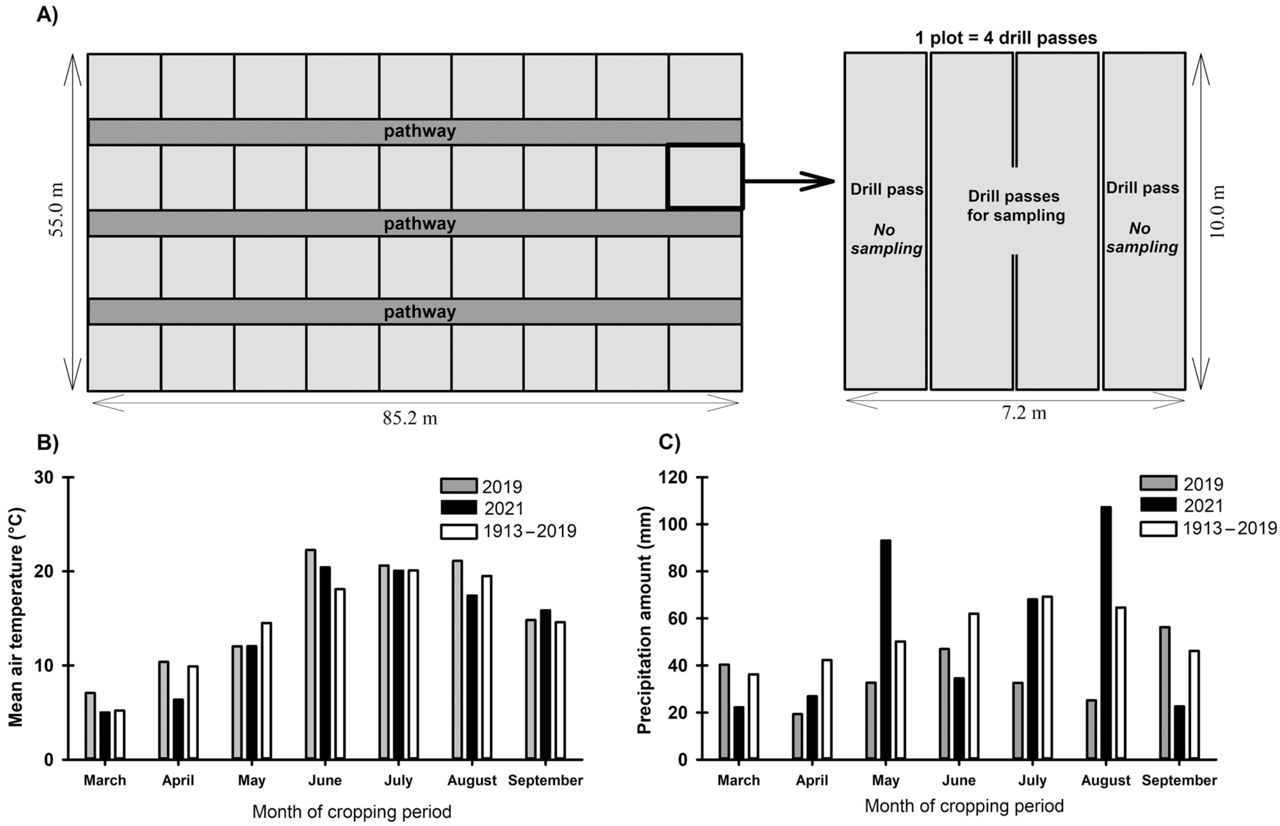

FIGURE 1 (A) Experimental design as a randomized block (85.2 m × 55 m) with each plot (7.2 m × 10 m) consisting of four drill passes. (B) Mean monthly temperature and long-term temperature mean (˚C) and (C) mean monthly precipitation and long-term mean of precipitation amount (mm) graphs for field trials at Canitz, Germany, from March to September 2019 and 2021.Weather data *source*: Wassergut Canitz GmbH.

significantly cooler at 9.8˚C (Figure 1B). In March 2019, the average monthly temperature was almost 2˚C above the long-term average. In April 2019, the average temperature remained in line with the long-term value, while April 2021 was 6.3˚C below the long-term average at 9.9˚C.

The trial site was characterized by low precipitation sums, averaging 557.3 mm per year and 370.7 mm for the March–September period (long-term means for the period 1913–2020), with total precipitation values of 253.4 mm in 2019 and 374.6 mm in 2021 (Figure 1C). May and August 2021 were exceptions, as there was 85.5% and 65.9% mm more precipitation compared to the long-term average for the period 1913–2020. The weather data were provided by the weather station at the Wassergut Canitz GmbH, farm in cooperation with Leipziger Stadtwerke.

Figure S1 captures the effect of climate change on daily temperature and precipitation values at the trial site from 2009 to 2023.

2.2 | Soil characterization and sampling

The site is characterized by Luvisols (para-brown soil) of periglacial gravel, with leading clay over deep sandy gravel. The soil texture is loamy sand (lS) (LfULG, 2020). The average pH value was 6.8, which was determined following the method described by VDLUFA (2012c). The available water capacity ranged from 121 to 180 mm, and the field capacity ranged from 181 to 270 mm. The soil compaction sensitivity level was medium (LfULG, 2020). The experimental field also showed a high sensitivity to water and a medium sensitivity to wind erosion (LfULG, 2020).

When the field trials were set up (April 1, 2019, and March 22, 2021), soil material was collected to analyze the plant-available phosphorus (P), plant-available potassium (K), and plant-available magnesium (Mg) contents at a depth of 0–0.3 m using a Pürckhauer soil auger (diameter 0.03 m, sampling length: 0.3 m). Sampling points were randomly selected throughout the trial site according to the recommendations for soil characterization of Lorenz and Erdle (2018). Plant-available in this study refers to the fraction of total P, K, and Mg contents in soil available for uptake by plant roots. The P and K contents were determined following the method described by VDLUFA (2012b) and amounted to 6.5 and 14.9 mg 100 g soil^{-1}, respectively, in 0- to 0.3-m soil depth. The Mg content was determined following the method of Walinga et al. (1995) and was 11.8 mg 100 g soil^{-1}, on average.

The target N content in soil for farms in water protection areas in Germany is 30 kg N ha^{-1} in order to reduce the nitrate levels in groundwater (Bundesministerium der Justiz und für Verbraucherschutz, 2009/22.12.2023). In the last 15 years, the farm has recorded an average surplus of 15 kg N ha^{-1} per year in the soil content for the subsequent crop. The N balance, including atmospheric N compounds, was between 15 and 20 kg ha^{-1} N surplus per year. NO_3^- levels in groundwater varied between 20 and 24 mg L^{-1}, which is below 25 mg L^{-1} (level at which anthropogenic effects are assumed) and 50 mg L^{-1} (limit value for groundwater in the European Union) (BMEL & BMU, 2020). The field trial area was not fertilized before sowing nor during the study period (2019–2021) to measure the effects on plant-available N in soil.

To determine the plant-available mineral N (N_{min}) content, samples were collected on the day of sowing at four depths at 0.3 m intervals (0–0.3, 0.3–0.6, 0.6–0.9, and 0.9–1.2 m) from the whole field trial area (Table S1). Samples for N_{min} determination were also collected on the first (June 27, 2019 and May 25, 2021), second (July 29, 2021), and last cutting dates (September 14, 2019, and September 14, 2021) at four depths, with four samples total along the plot width. Soil samples were taken on 2 days in 2019 and 8 days in 2021 after the last hand harvest of plant material and before plow-up. There are no N_{min} or nitrate values at later points in autumn provided in this manuscript, as this work focuses on the effect of the mixtures on soil N_{min} during the cultivation period. The soil material of each subsample and the same interval were mixed, and stones and plant material were removed. On the site, the samples were stored and transported in cool boxes at about 5˚C. Afterward, they were stored at −18˚C until NO_3^- and NH_4^+ extraction. The detailed approach for NO_3^- and NH_4^+ analysis was described by Krachunova et al. (2023). To calculate the N_{min} content in the soil, the dry matter of the soil material was determined in a drying cabinet at 105˚C for 48 h. The calculations were conducted according to the following formula taken from VDLUFA (2012a) and Deutsches Institut für Normung e.V (DIN) (2005–2006):

$$\mathrm{N\,min} = \frac{\mathrm{NH_4} + \left(\mathrm{mg\,L^{-1}}\right) \times d\ (\mathrm{dm}) \times \mathrm{dB}\ \left(\mathrm{g\,cm^{-3}}\right)}{\mathrm{DM\ soil}\ \times f\ \left(\mathrm{mL\ g^{-1}}\right)} + \frac{\mathrm{NO_3} -\ (\mathrm{mg\,L^{-1}}) \times d\,(\mathrm{dm}) \times \mathrm{dB}\ \left(\mathrm{g\,cm^{-3}}\right)}{\mathrm{DM\,soil} \times f\ (\mathrm{mL\ g^{-1}})} \tag{1}$$

where d is soil depth interval (0.3 m); dB is soil density in natural storage, including pore space (1.55 g cm^{-3}); f is $CaCl_2$ concentration per FM (fresh matter)/soil material (2.50 mL g^{-1}).

The value of dB (soil density in natural storage, including pore space) was provided by the laboratory of the State Operating Company for Environment and Agriculture (BfUL) in Nossen, Free State of Saxony, Germany.

2.3 | Plant cultivars and sowing

The seed density of the pure stand treatments (100%) was 400 pure live seeds per m^{-2} with a 0.13 m row spacing. Cultivars La Bella Campagnola (alfalfa), Libor (ribwort plantain), and Cosmolit (meadow fescue) of certified organic quality were used for both field trials in 2019 and 2021. La Bella Campagnola is a Mediterranean cultivar with high drought tolerance. The ribwort plantain cultivar Libor contains around 2% aucubin and can grow from semi-shade to no direct sunlight, according to the plant breeder Jelitto Staudensamen GmbH. Cosmolit is a drought-resistant cultivar typically used for alfalfa mixtures in Germany (Bundessortenamt, 2020).

Prior to sowing, the soil was prepared using a turning plow (Lemken, at 0.27-m depth) and a cultivator (Maschio S.p.A, at approximately 0.08-m depth). All seeds were deposited on the soil surface (Seed drill Wintersteiger Hege 80/Kombi) on April 1, 2019, and February 28, 2021. The soil surface was flattened with a roller (Prismatic roller Mediana, Güttler) after seeding. The cropping period ended on October 17, 2019, and October 21, 2021, when the fields were plowed.

2.4 | Plant sampling

Aboveground plant material was harvested on three dates: at the late bud stage of alfalfa (June 27, 2019, and May 25, 2021), at alfalfa early flowering on July 29, 2021, and at the alfalfa late flowering stage of both alfalfa and ribwort plantain (October 14, 2019, and September 14, 2021). No second cutting of plant material was possible in the 2019 field trial at the early flowering stage of alfalfa due to heavy weed infestation. Instead, the area was mowed in July 2019. Each harvest consisted of manually cutting 1.5 m^2 of fresh matter with hedge shears, which was then used to determine the species composition. Forage accumulation (FA) was then determined by harvesting a 7 m^{-2} strip from the center of each plot with a manually operated green fodder plot harvester (Haldrup GmbH). The material was collected immediately after cutting with rakes and the fresh matter was weighed in the field (Bosche Wägetechnik, 0.2 ± 60.0 kg). Dry matter was determined by drying until attaining constant weight at 105˚C (48 h, Thermo UT 6760, +20 up to 300˚C, Heraeus Holding GmbH).

The hand-cut samples were kept under cool conditions overnight (5˚C) until sample processing took place the next day. Each sample was separated to quantify the mixture species fractions and weed biomass. The weeds present at the study site were assessed with the visual counting system for weed density called Göttinger Zahl- und Schätzrahmen as described by LfL (2024) on the same day of hand harvest. The fresh weights of the whole sample and of the individual forage mixture components were then determined (Denver Instrument SI-6002, accuracy ± 0.01 g).

2.5 | Statistical analysis

All statistical analyses were implemented with SAS statistical software (version 9.4 for Windows, SAS Institute Inc.). Preliminary examination of the raw datasets revealed that the FA varied greatly between the 2 years of study. Consequently, differences in the N_{min} distribution during preliminary analyses were also discovered. For this reason, the authors carried out two separate analyses of each year to represent all the effects of the mixture treatments.

All data were analyzed using PROC GLM (general linear model) for both one (treatments T mixtures and pure stands) and multi-factor (variables years 2019 and 2021 (Y), T and Y × T) analysis of variance. PROC GLM with the least square (LS) method was chosen for the block design of the field trial as recommended by Munzert (2015). The conservative approach of Tukey's honestly significant difference (HSD, *p*-level set at the level as the largest mean difference) post hoc test was used for specific pairwise comparisons (Tukey, 1949). Normal distribution of all datasets was tested with the Shapiro–Wilk test (Shapiro & Wilk, 1965), whereby non-normally distributed data were transformed according to the guidelines of Köhler et al. (2012) and Munzert (2015), the functions 1:x, $\sqrt{(x)}$, x^2, $\sqrt{(1{:}x)}$,$1{:}x^2$, and log(x). The statistics of the data as well as the presentation were carried out with the arithmetic mean and standard deviation of the mean. The significance level for all presented results was $p \leq 0.05$.

The metric unit tonnes (t) was used for the FA results and was calculated generally as follows:

$$\text{t ha} = \frac{\text{DM yield sample (g)}}{1.5\text{m}^2 \text{ harvested plot area}} \times 100 \tag{2}$$

where g represents metric gram and m^2 represents metric square meter.

The total FA corrected for DM (dry matter) (t ha^{-1}) was used for the calculation of the partial land equivalent ratio (pLER) and total land equivalent ratio (LER). The LER was determined following the equation given by Willey and Osiru (1972) and was calculated as follows:

$$\text{Partial LER} = \frac{\text{Forage accumulation of mixture component 1}}{\text{Forage accumulation of pure stand crop species 1}} \tag{3}$$

$$\text{Total LER} = \frac{\text{Forage accumulation of mixture component species 1}}{\text{Forage accumulation of pure stand crop species 1}} + \frac{\text{Forage accumulation of mixture componen species 2}}{\text{Forage accumulation of pure stand crop species 2}} \tag{4}$$

Significant effects of the mixture interaction between the replacement series were identified. Graphical illustrations of the results were generated with SigmaPlot (Version 12.5, Systate Software Inc.).

3 | RESULTS

3.1 | Forage accumulation and competition development

All treatments showed very low dry matter yields at the first cut in both trial years, with alfalfa showing the lowest values ($p = 0.01$, Table 2). The FA from treatments with forage mixtures was greater than that of alfalfa in both trial years (Table 2). Ribwort plantain with alfalfa in 50/50% mixtures led to greater FA in the first cut in 2019 compared to alfalfa in either year with all other treatments being intermediate to but not different from these two treatments. In the second cut in 2021, ribwort plantain in all alfalfa mixtures produced the greatest FA numerically, although the increase was not different from ribwort plantain, 33/67% alfalfa with meadow fescue, or 50/50% alfalfa with meadow fescue. Alfalfa had the lowest FA at the third cut in 2019 at 0.28 t ha^{-1}, which was lower than all alfalfa and ribwort plantain mixtures and ribwort plantain and meadow fescue in pure stands ($p < 0.0001$). Meadow fescue showed greater FA than in the mixtures with alfalfa. No statistical differences among treatments were determined at the third cut of 2021 ($p = 0.59$). Total FA showed similar trends in 2019 and 2021, in which ribwort plantain and ribwort plantain/alfalfa mixtures generally produced greater FA compared to alfalfa in pure stands and meadow fescue mixtures.

The most dominant weed was common poppy (*Papaver rhoeas* L.), with over 85% occurrence. The other 15% of weed occurrences consisted of purple deadnettle (*Lamium purpureum* L.), cornflower (*Centaurea cyanus* L.), common dandelion (*Taraxacum officinale* L.), common chickweed (*Stellaria media* L.), common amaranth (*Amaranthus retroflexus* L.), and single plants of red clover (*Trifolium pratense* L.), jointed charlock (*Raphanus raphanistrum* L.), and common bugloss (*Anchusa officinalis* L.) spread throughout the whole trial field.

Ribwort plantain significantly suppressed alfalfa in mixture treatments 50/50% and 67/33% at the first cut in both

TABLE 2 Forage accumulation (FA) (t ha^{-1}) of pure stands and mixture treatments of plantain, alfalfa, and meadow fescue in field trials in 2019 and 2021. The cuts were conducted as follows: 1. Cut at the late bud stage of alfalfa (June 27, 2019, and May 25, 2021), 2. cut at early alfalfa flowering (July 29, 2021; data not available for 2019), and 3. cut at the late flowering stage of alfalfa and ribwort plantain (October 14, 2019, and September 14, 2021). One-factor analysis for 2019 and 2021, and multiple comparison for 2019 × 2021 with Tukey's honestly significant difference (HSD). *p*-values indicate significance within one column of the same cut.

Year	Treatment	Seed rate (%)	1. Cut FA (t ha^{-1})	2. Cut FA (t ha^{-1})[a]	3. Cut FA (t ha^{-1})	Total FA (t ha^{-1})
2019	R. plantain	100	0.06ab	X	1.61a	1.67a
	Alfalfa/R. plantain	33/67	0.05ab	X	1.55a	1.61a
		50/50	0.09a	X	1.69a	1.78a
		67/33	0.05ab	X	1.12ab	1.17ab
	Alfalfa	100	0.01b	X	0.28c	0.29c
	Alfalfa/meadow fescue	33/67	0.03ab	X	0.71bc	0.75bc
		50/50	0.03ab	X	0.71bc	0.74bc
		67/33	0.03ab	X	0.68bc	0.71bc
	Meadow fescue	100	0.04ab	X	1.19ab	1.23ab
	Tukey-HSD *p*-values		**0.01**	X	**<0.0001*****	**<0.0001*****
2021	R. plantain	100	0.15ab	4.51abc	1.43	6.09abc
	Alfalfa/R. plantain	33/67	0.18ab	6.62a	1.52	8.32a
		50/50	0.27a	6.19a	1.79	8.25a
		67/33	0.16ab	5.14ab	1.37	6.67ab
	Alfalfa	100	0.01b	2.16c	1.61	3.78bc
	Alfalfa/meadow fescue	33/67	0.04b	2.13abc	1.56	3.73c
		50/50	0.10ab	2.44abc	1.48	4.02bc
		67/33	0.11ab	1.68c	1.89	3.68c
	Meadow fescue	100	0.11ab	2.05c	1.94	4.11bc
	Tukey-HSD *p*-values		**0.01****	**<0.0001*****	**0.59**[ns]	**<0.0001*****
2019 × 2021	**Multiple comparison *p*-values**	**Y**	**<0.0001*****	X	**<0.0001*****	**<0.0001*****
		T	**0.001*****	X	**0.001*****	**<0.0001*****
		Y × T	**0.15**[ns]	X	**0.001**	**0.001**

Note: Data for multiple comparisons are weighted means of four replications per year (Y) and treatment (T). Letters following values indicate significant differences within the same column according to a multiple comparison test. Seed rate (%) column—numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent.
Abbreviations: ns, not significant; R. plantain, ribwort plantain; T, treatment; Y, year; Y × T, interactive effect between years and treatments.
[a]X's indicate daata available only for 2021; one-factor analysis with Tukey-HSD.
**, ***Significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

field trials ($p < 0.0001$, Table 3). Alfalfa did not establish very well at the first cut in 2019 or 2021, but it generally showed higher shares in all treatments with meadow fescue. Alfalfa showed the significantly lowest share (18.9%) compared to ribwort plantain (81.1%) in the 67/33% treatment ($p < 0.0001$, Table 3). Alfalfa had higher shares in ribwort plantain and meadow fescue mixture treatments during 2019 ($p_T < 0.0001$), under drier conditions, compared to 2021, which led to significant interactions between year and treatment in the multiple comparison ($p_{Y \times T} = 0.001$, Table 3).

In the second cut in 2021, the highest share of alfalfa amounted to 27.7% in the 67/33% meadow fescue treatment ($p < 0.0001$). Significantly lower shares of alfalfa were found in the 33/67% and 50/50% ribwort plantain mixtures. The reduction in alfalfa share in the 33/67% ribwort plantain mixture was drastic, from 48.9% during the first cut to 3.1% in the second cut (Table 3).

The share of alfalfa decreased at the third cut in 2019 and 2021. The highest share of alfalfa was in the 67/33% mixture with meadow fescue in 2019 (11.4%). Alfalfa at the third cut in 2019 and 2021 was generally far below shares of ribwort plantain and meadow fescue in all treatments ($p < 0.0001$, Table 3). The highest share of ribwort plantain was 89.5% in 2019 and 95.1% in 2021 (Table 3).

3.2 | pLER and LER

The highest pLERs for alfalfa and ribwort plantain in 2019 and 2021 were in the 50/50% mixture, but significant only in 2019 ($p = 0.002$, Table 4). As a result, the highest total LERs in the same treatment were significant in 2019 ($p = 0.01$, Table 4).

TABLE 3 Competition development in the share of alfalfa, ribwort plantain, and meadow fescue (%) in the mixture treatments in field trials in 2019 and 2021. The cuts were conducted as follows: 1. Cut at the late bud stage of alfalfa (June 27, 2019, and May 25, 2021), 2. cut at early alfalfa flowering (July 29, 2021; data not available for 2019), and 3. cut at the late flowering stage of alfalfa and ribwort plantain (October 14, 2019, and September 14, 2021). One-factor analysis for 2019 and 2021, and multiple comparison for 2019 × 2021 with Tukey's honestly significant difference (HSD). *p*-values indicate significance within two columns of the same cut.

Year	Treatment	Seed rate (%)	1. Cut alfalfa share (%)	1. Cut R. plantain/ M. fescue share (%)	2. Cut alfalfa share (%)[a]	2. Cut R. plantain/ M. fescue share (%)[a]	3. Cut alfalfa share (%)	3. Cut R. plantain/ M. fescue share (%)	Mean Alfalfa share (%)	Mean R. plantain/ M. fescue share (%)
2019	Alfalfa/R. plantain	33/67	28.33cd	71.67ab	X	X	1.34d	98.66a	14.84b	85.16a
		50/50	28.32cd	71.68ab	X	X	1.17d	98.83a	14.74b	85.26a
		67/33	18.88d	81.12a	X	X	2.08d	97.92a	10.48b	89.52a
	Alfalfa/meadow fescue	33/67	41.28bcd	58.72abc	X	X	2.22d	97.78a	21.75b	78.25a
		50/50	43.48abcd	56.52abcd	X	X	9.49c	90.51b	26.49b	73.51a
		67/33	47.77abcd	52.23abcd	X	X	11.37c	88.63b	29.57b	70.43a
	Tukey-HSD *p*-values		**<0.0001*****		X		**<0.0001*****		**<0.0001*****	
2021	Alfalfa/R. plantain	33/67	48.90ab	51.10ab	3.10d	96.90a	2.99b	97.01a	18.33b	81.67a
		50/50	6.59b	93.41a	4.29d	95.71a	3.92b	96.08a	4.93b	95.07a
		67/33	12.50b	87.50a	15.58cd	84.42ab	10.58b	89.42a	12.89b	87.11a
	Alfalfa/meadow fescue	33/67	39.53ab	60.47ab	12.55cd	87.45ab	4.72b	95.28a	18.93b	81.07a
		50/50	20.58b	79.42a	15.88cd	84.12ab	7.47b	92.53a	14.65b	85.35a
		67/33	3.96b	96.04a	27.73c	72.27b	10.25b	89.75a	13.98b	86.02a
	Tukey-HSD *p*-values		**<0.0001*****		**<0.0001*****		**<0.0001*****		**<0.0001*****	
2019 × 2021	**Multiple comparison *p*-values**	**Y**	**1.0**[ns]		X		**1.0**[ns]		**1.0**[ns]	
		T	**<0.0001*****		**<0.0001*****		**<0.0001*****		**<0.0001*****	
		Y × T	**0.001**		X		**0.01**		**0.01**	

Note: Data in the multiple comparisons are weighted means of four replications per year (Y) and treatment (T). Letters following values indicate significant differences within the same column according to a multiple comparison test. Seed rate (%) column—Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent.

Abbreviations: ns, not significant; M. fescue, meadow fescue; R. plantain, ribwort plantain; T, treatment; Y, year; Y × T, interactive effect between years and treatments.

[a]X's indicate data available only for 2021, one-factor analysis with Tukey-HSD.

***Significant at $p \leq 0.01$ and 0.001.

TABLE 4 Partial land equivalent ratio (pLER) and total land equivalent ratio (total LER) of ribwort plantain, alfalfa, and meadow fescue in the mixture treatments in field trials in 2019 and 2021. The cuts were conducted as follows: 1. Cut at the late bud stage of alfalfa (June 27, 2019, and May 25, 2021), 2. cut at early alfalfa flowering (July 29, 2021; data not available for 2019), and 3. cut at the late flowering stage of alfalfa and ribwort plantain (October 14, 2019, and September 14, 2021). One-factor analysis for 2019 and 2021, and multiple comparison for 2019 × 2021 with Tukey's honestly significant difference (HSD). *p*-values indicate significance within two columns of the pLER for the same cut. *p*-values indicate significance within the total LER from the same cut.

Year	Treatment	Seed rate (%)	1. Cut pLER Alfalfa	1. Cut pLER R. plantain/ M. fescue	1. Cut Total LER	2. Cut pLER Alfalfa[a]	2. Cut pLER R. plantain/M. fescue	2. Cut Total LER[a]	3. Cut pLER Alfalfa	3. Cut pLER R. plantain/ M. fescue	3. Cut Total LER	Total pLER Alfalfa	Total pLER R. plantain/ M. fescue	Total LER
2019	Alfalfa/R. Plantain	33/67	0.86abc	0.74abc	1.60b	X	X	X	0.06b	1.01a	1.07	0.57b	0.88ab	1.45ab
		50/50	1.72a	1.15abc	2.87a	X	X	X	0.07b	1.07a	1.14	1.59a	1.11ab	2.70a
		67/33	1.51ab	0.65bc	2.16ab	X	X	X	0.10b	0.74ab	0.84	0.57b	0.69b	1.26b
	Alfalfa/meadow fescue	33/67	1.02abc	0.49bc	1.51b	X	X	X	0.11b	0.63ab	0.74	0.78ab	0.56b	1.34ab
		50/50	1.12abc	0.45bc	1.57b	X	X	X	0.27ab	0.57ab	0.84	0.70b	0.51b	1.21b
		67/33	0.99abc	0.36c	1.35b	X	X	X	0.58ab	0.55ab	1.13	0.79ab	0.45b	1.24b
	Tukey-HSD *p*-values		**0.002****		**0.01****	X		X	**0.001*****		**0.52**	**0.003****		**0.01****
2021	Alfalfa/R. plantain	33/67	1.41	0.65	2.06	0.09e	1.42a	1.52	0.04d	1.07a	1.11	0.68bc	1.05ab	1.73
		50/50	1.52	1.63	3.15	0.12e	1.32ab	1.44	0.04d	1.23a	1.27	0.56bc	1.39a	1.95
		67/33	0.82	0.92	1.74	0.36de	0.97abc	1.33	0.08c	0.87abc	0.95	0.42bc	0.92abc	1.34
	Alfalfa/meadow fescue	33/67	1.50	0.50	2.00	0.17e	0.64cde	0.81	0.04d	0.83abcd	0.87	0.45bc	0.66bc	1.11
		50/50	1.77	0.43	2.20	0.31de	0.86bcd	1.17	0.06d	0.73abcd	0.79	0.72bc	0.68bc	1.40
		67/33	0.45	0.92	1.37	0.53cde	0.48cde	1.01	0.13bcd	0.91ab	1.04	0.37c	0.77abc	1.14
	Tukey-HSD *p*-values		**0.2**[ns]		**0.48**[ns]	**<0.0001*****		**0.15**[ns]	**<0.0001*****		**0.59**[ns]	**0.001*****		**0.17**[ns]
2019 × 2021	***p*-values**	**Y**	**0.32**[ns]		**0.18**[ns]	X		X	**0.63**[ns]		**0.57**[ns]	**0.5**[ns]		**0.34**[ns]
		T	**0.001*****		**0.1**[ns]	**<0.0001*****		**0.15**[ns]	**<0.0001*****		**0.23**[ns]	**0.001*****		**0.01**
		Y × T	**0.49**[ns]		**0.98**[ns]	X		X	**0.4**[ns]		**0.96**[ns]	**0.01**		**0.89**[ns]

Note: Data for the multiple comparisons are weighted means of four replications per year (Y) and treatment (T). Letters following values indicate significant differences within the same column according to a multiple comparison test.

Abbreviations: ns, not significant; M. fescue, meadow fescue; R. plantain; ribwort plantain; T, treatment; Y, year; Y × T, interactive effect between years and treatments.

[a]X's indicate data available only for 2021, one-factor analysis with Tukey-HSD. Seed rate (%) column—Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent.

***,**Significant at $p \leq 0.01$ and 0.001, respectively.

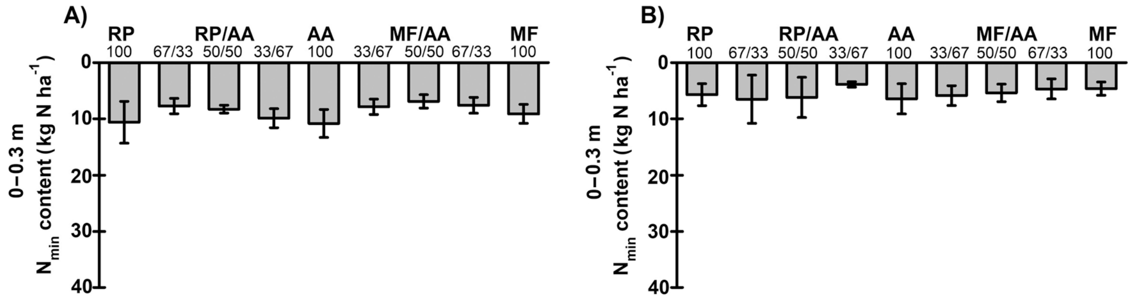

FIGURE 2 Plant-available mineral nitrogen (N_{min}) content (kg N ha^{-1}) at a 0- to 0.3-m soil depth in field trials in 2019 and 2021: (A) on June 27, 2019, $p = 0.06$; and (B) on May 25, 2021, $p = 0.66$. One-factor analysis with Tukey's honestly significant difference (HSD). Means ± standard deviation. Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent, and 100 indicates a pure stand. AA, alfalfa pure stand; MF, meadow fescue; MF/AA, meadow fescue/alfalfa mixtures; RP, ribwort plantain pure stand; RP/AA, ribwort plantain/alfalfa mixtures.

There were no significances in 2021 (pLER $p = 0.2$, total LER $p = 0.48$, Table 4). For both pLER and total LER, there were no significant interactions between year and treatment (pLER $p_{Y \times T} = 0.49$, total LER $p_{Y \times T} = 0.98$, Table 4).

For the second cut in 2021, a significant decline in the pLER of alfalfa was observed in all treatments ($p < 0.0001$, Table 4). pLER of ribwort plantain was 33/67% and was significantly higher than all other pLERs ($p < 0.0001$, Table 4). The total LER in the second cut was numerically higher among alfalfa/ribwort plantain than alfalfa/meadow fescue treatments in 2021, but not significant ($p = 0.15$, Table 4).

In the third cut in 2019 and 2021, the highest pLER was achieved by ribwort plantain in the 50/50% treatment with alfalfa ($p = 0.001$ in 2019 and $p < 0.0001$ in 2021, Table 4). The pLER values of alfalfa were significantly lower than the pLER of ribwort plantain in 33/67% and 50/50% mixtures in 2019 and 2021 ($p = 0.001$ in 2019, $p < 0.001$ in 2021, Table 4). The total LER of the treatments showed no significances in the one-factor analysis or significant interactions in the multiple comparison ($p = 0.52$ in 2019, $p = 0.52$ in 2021, $p_{Y \times T} = 0.96$, Table 4).

Total LER over three cuts was significantly highest for 50/50% alfalfa/ribwort plantain treatments in 2019 ($p = 0.01$, Table 4). There were no significant differences for the total LER in 2021 ($p = 0.17$, Table 4). Overall, a higher total LER without significant interactions of year × treatment was achieved under the ribwort plantain treatments across all cuts and years ($p_T = 0.01$, $p_{Y \times T} = 0.89$, Table 4).

3.3 | N_{min} content

At 0- to 0.3-m soil depth, no clear trends in N_{min} development were observed for the first cut in either trial year (Figure 2A,B). Higher values were recorded in 2019 for ribwort plantain (10.6 kg N ha^{-1}) and alfalfa (10.8 kg N ha^{-1}) in pure stands, but not significantly ($p = 0.06$, Figure 2A). In 2021, the 67/50% and 50/50% ribwort plantain/alfalfa mixtures, as well as alfalfa in pure stands, showed the greatest standard deviations and no significance ($p = 0.66$, Figure 2B).

On the autumn date in 2019, N_{min} of 100% alfalfa showed significant differences at 0- to 0.3-m depth from 100% ribwort plantain and 67/33% ribwort plantain/alfalfa treatments ($p = 0.03$, Figure 3A). The significantly highest N_{min} at 0.3- to 0.6-m soil depth was determined under 100% alfalfa in 2019 and there was a trend for higher N_{min} in meadow fescue/alfalfa treatments ($p = 0.01$, Figure 3B). Significant differences among the N_{min} were also determined from 0.6 to 0.9 m in 2019, with 100% meadow fescue having the highest mean ($p = 0.01$, Figure 3C). From 0.9 to 1.2 m, the trend for higher N_{min} content under 100% alfalfa and alfalfa/meadow fescue treatments remained visible, but without a significance ($p = 0.16$, Figure 3D).

In autumn 2021, the N_{min} values under the presence of ribwort plantain, to the left of pure-cultured alfalfa in Figure 4A showed almost equal N_{min} values with a low standard deviation, while the values for alfalfa in pure stands and meadow fescue mixtures showed a large dispersion. There were no significances, as shown in Figure 4A ($p = 0.11$). The highest N_{min} value in 2021 was also found at 0.3–0.6 m, as in 2019, under 100% alfalfa ($p = 0.03$, Figure 4B). The 50/50% and 33/67% ribwort plantain/alfalfa mixtures showed significantly lower N_{min} contents. From 0.6- to 0.9-m depth, the N_{min} values showed a greater scatter, with 33/67% meadow fescue/alfalfa treatment having the highest value. The lowest value was found under 100% ribwort plantain, but not significant ($p = 0.07$, Figure 4C). From 0.9 to 1.2 m, the individual values of the treatments were scattered and not significant, but higher N_{min} values were found with meadow fescue/alfalfa treatments ($p = 0.4$, Figure 4D).

3.4 | NO_3-N and NH_4-N share

Analysis of the mean values showed that the significantly lowest NO_3-N share (65.9%) was present in the pure stand

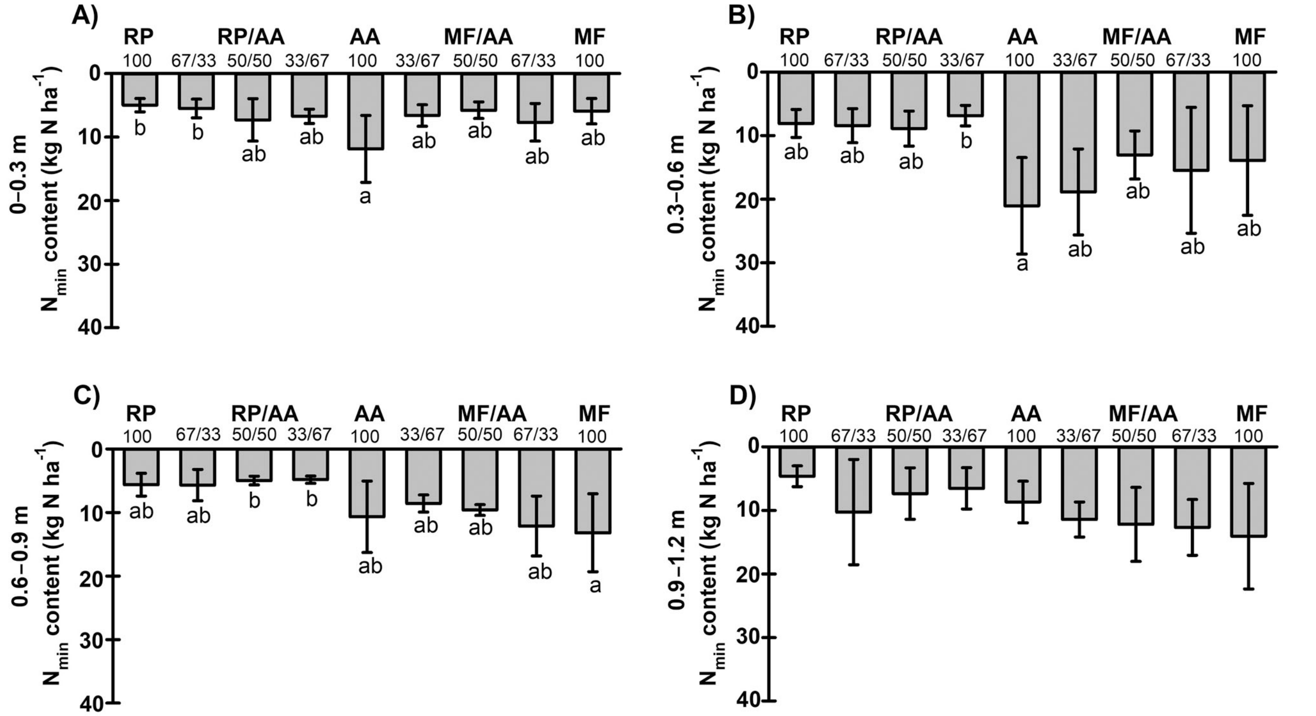

FIGURE 3 Plant-available mineral nitrogen (N_{min}) content (kg N ha^{-1}) at different soil depths in the 2019 field trial on October 16, 2019: (A) 0–0.3 m, $p = 0.03$; (B) 0.3–0.6 m, $p = 0.01$; (C) 0.6–0.9 m, $p = 0.01$; (D) 0.9–1.2 m, $p = 0.16$. One-factor analysis with Tukey's honestly significant difference (HSD). Means ± standard deviation. Lowercase letters a and b indicate significance within the same sub-figure. Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent, and 100 indicates a pure stand. AA, alfalfa pure stand; MF, meadow fescue; MF/AA, meadow fescue/alfalfa mixtures; RP, ribwort plantain pure stand; RP/AA, ribwort plantain/alfalfa mixtures.

of ribwort plantain (p = 0.02, Figure 5A). The 33/67% ribwort plantain/alfalfa treatment showed the significantly highest proportion of NO_3-N (85.6%) at 0–0.3 m. Conversely, 100% ribwort plantain had the significantly highest NH_4-N share in pure stands and the significantly lowest share in the 33/67% treatment with alfalfa. No significant differences were observed from 0 to 0.3 m at the first cutting date in May 2021 (p = 0.76, Figure 5B). In contrast to the cut in 2019, 100% alfalfa showed the lowest NO_3-N content of all treatments. Ribwort plantain and meadow fescue in pure stands showed similar values (Figure 5B).

There were no significant differences in NO_3-N and NH_4-N from 0- to 0.3-m depth during the second cut in mid-October 2019 (p = 0.14, Figure 6A). However, the treatments with 33/67% and 50/50% meadow fescue/alfalfa had the lowest proportion of NO_3-N and consequently the highest proportion of NH_4-N. At 0.3- to 0.6-m soil depth, the 67/33% ribwort plantain/alfalfa treatment had the significantly lowest NO_3-N ratio and highest NH_4-N ratio compared to 100% alfalfa and 33/67% meadow fescue/alfalfa treatment (p = 0.01, Figure 6B). From 0.6- to 0.9-m depth, the NO_3-N proportion was higher in 100% alfalfa and meadow fescue and all meadow fescue/alfalfa treatment than in 100% ribwort plantain, but not significant (p = 0.1, Figure 6C). The highest NO_3-N shares occurred in 33/67% and 50/50% alfalfa/meadow fescue mixtures. The NH_4-N percentage in pure ribwort plantain was slightly lower compared to all ribwort plantain/alfalfa treatments (Figure 6C). At 0.9- to 1.2-m depth, the NH_4-N ratio decreased in all treatments, except for the 67/33% ribwort plantain/alfalfa mixture. There were outliers in the datasets for this and 67/33% meadow fescue/alfalfa treatments, which is why it was difficult to interpret the results (p = 0.16, Figure 6D).

At the second cutting date in mid-September 2021, the NO_3-N share at 0–0.3 m was significantly higher with 100% alfalfa and 33/67% meadow fescue/alfalfa in contrast to the 33/67% ribwort plantain/alfalfa treatment (p = 0.04, Figure 7A). From 0.3 to 0.6 m, the significantly lowest NO_3-N share was observed with pure ribwort plantain (p = 0.003, Figure 7B). Pure cultured alfalfa and the 50/50% meadow fescue/alfalfa treatments had similar values from 0.3 to 0.6 m (Figure 7B). From 0.6 to 0.9 m, the difference between the NO_3-N /NH_4-N ratio with pure alfalfa and the 33/67% meadow fescue/alfalfa mixture clearly increased, but without a significance (p = 0.09, Figure 7C). With 100% alfalfa, the NO_3-N share was 37.4% higher than in the 33/67% ribwort plantain/alfalfa mixture compared with 0.3–0.6 m. Furthermore, Figure 7C reveals that all treatments containing ribwort plantain (left side of 100% alfalfa in Figure 7C) had higher NH_4-N shares than all treatments with meadow

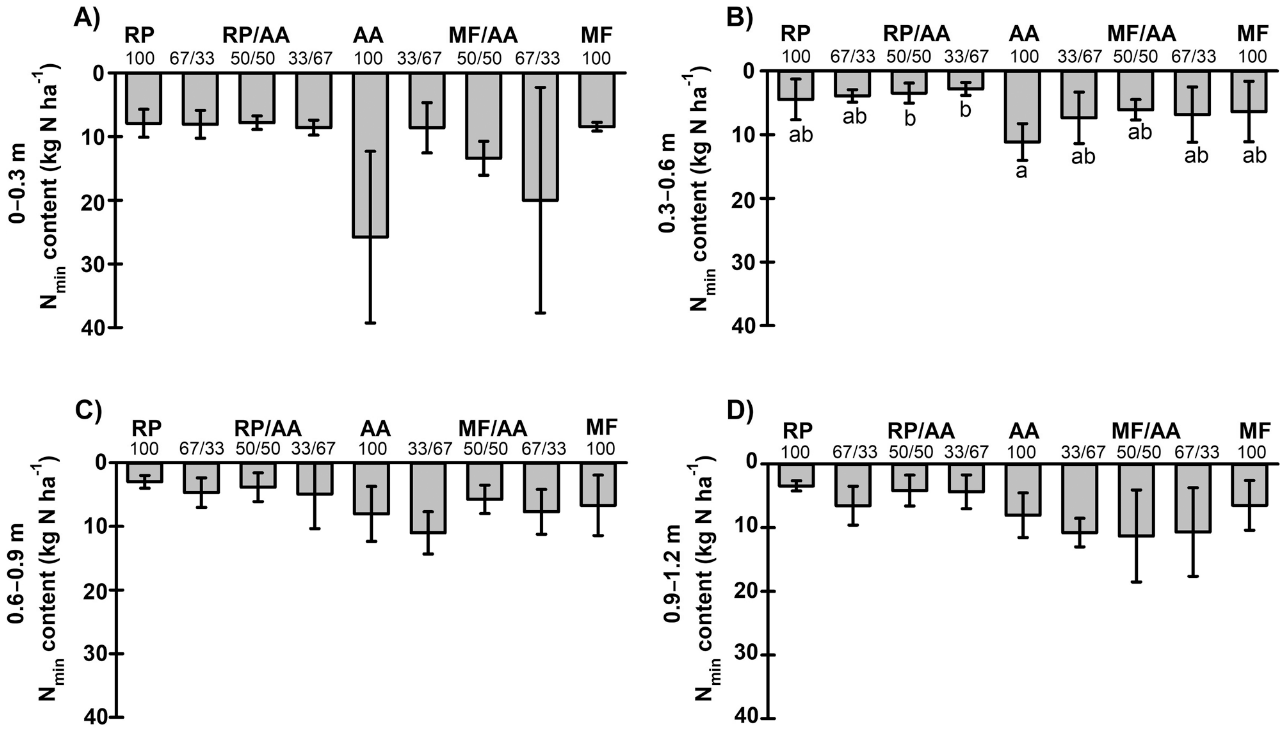

FIGURE 4 Plant-available mineral nitrogen (N_{min}) content (kg N ha^{-1}) at different soil depths in the 2021 field trial on September 14, 2021: (A) 0–0.3 m, $p = 0.11$; (B) 0.3–0.6 m, $p = 0.03$; (C) 0.6–0.9 m, $p = 0.07$; and (D) 0.9–1.2 m, $p = 0.4$. One-factor analysis with Tukey's honestly significant difference (HSD). Means ± standard deviation. Lowercase letters a and b indicate significance within the same sub-figure. Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent, and 100 indicates a pure stand. AA, alfalfa pure stand; MF, meadow fescue; MF/AA, meadow fescue/alfalfa mixtures; RP, ribwort plantain pure stand; RP/AA, ribwort plantain/alfalfa mixtures.

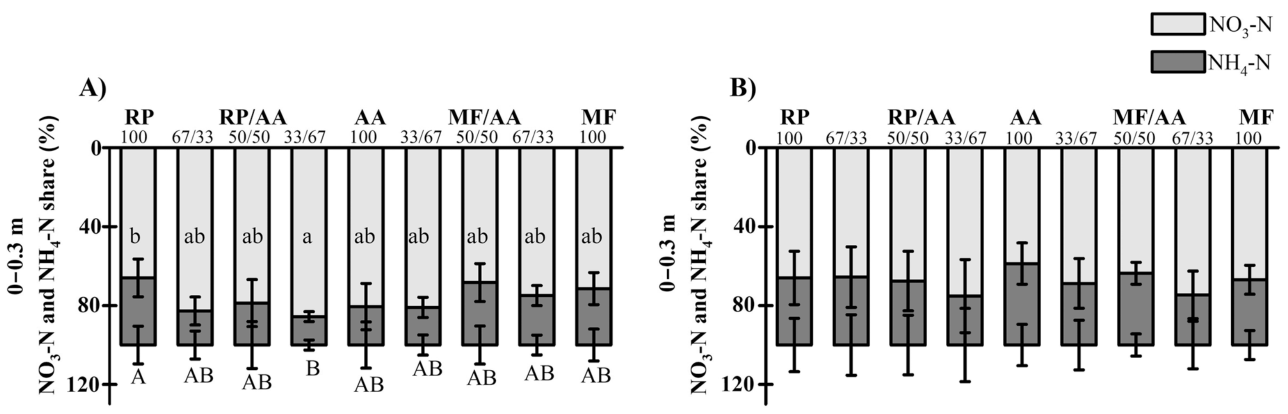

FIGURE 5 NO_3-N to NH_4-N (%) share in total N_{min} at a 0- to 0.3-m soil depth in field trials in 2019 and 2021: (A) 0–0.3 m on June 27, 2019, $p_{NO_3\text{-N}} = 0.02$, $p_{NH_4\text{-N}} = 0.02$; and (B) 0–0.3 m on May 25, 2021, $p_{NO_3\text{-N}} = 0.76$, $p_{NH_4\text{-N}} = 0.76$. One-factor analysis with Tukey's honestly significant difference (HSD). Means ± standard deviation. Lowercase letters a and b indicate the significance of NO_3-N (%) within the same sub-figure. Uppercase letters A and B indicate the significance of NH_4-N (%) within the same sub-figure. Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent, and 100 indicates a pure stand. AA, alfalfa pure stand; MF, meadow fescue; MF/AA, meadow fescue/alfalfa mixtures; RP, ribwort plantain pure stand; RP/AA, ribwort plantain/alfalfa mixtures.

fescue (right of 100% alfalfa in Figure 7C). From 0.9 to 1.2 m, the NO_3-N shares increased in the 33/67% and 50/50% meadow fescue/alfalfa mixtures and were significantly higher than 67/33% and 33/67% ribwort plantain/alfalfa mixtures ($p = 0.001$, Figure 7D).

4 | DISCUSSION

The authors showed for the first time that a ribwort plantain/alfalfa mixture increased the share of NH_4-N in annual alfalfa mixtures in an autumn plowing. Although the effect

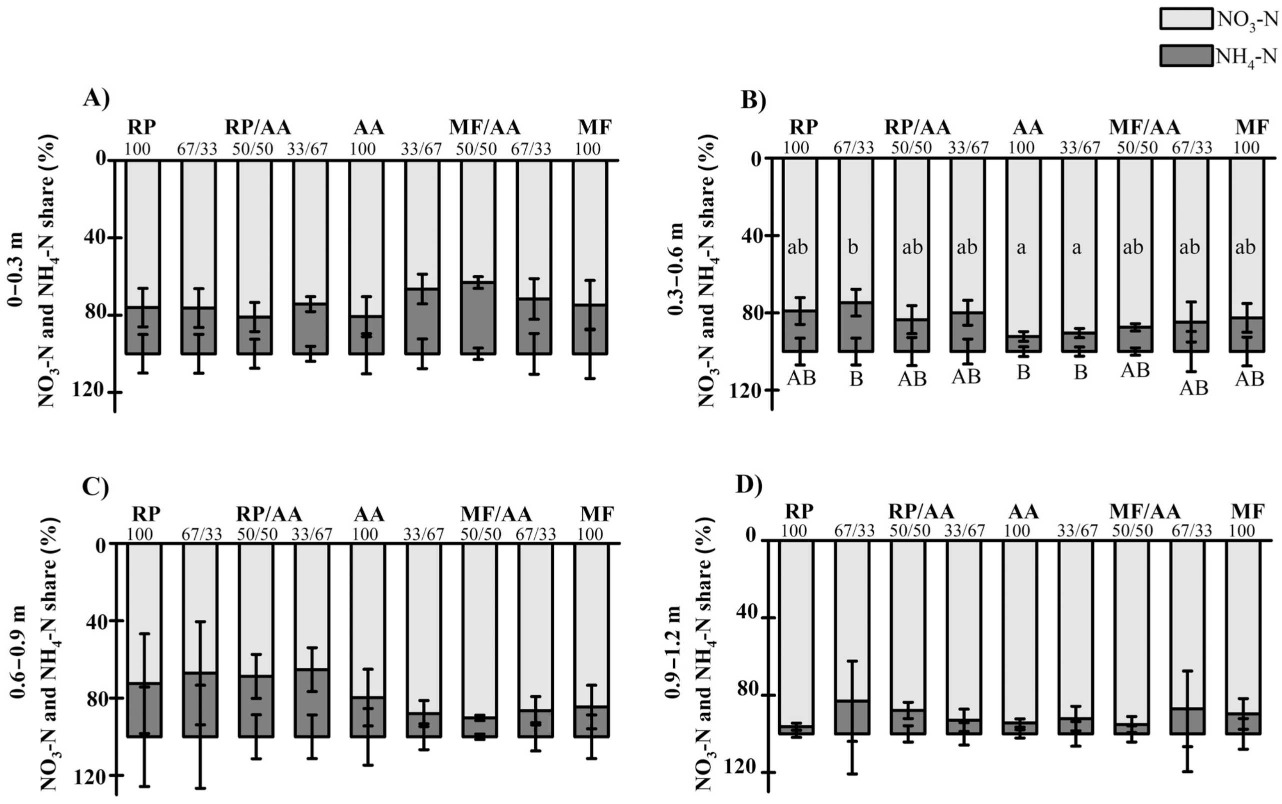

FIGURE 6 NO_3-N to NH_4-N (%) share in total N_{min} at different soil depths in the 2019 field trial on October 16, 2019: (A) 0–0.3 m, $p_{NO_3\text{-}N} = 0.14$, $p_{NH_4\text{-}N} = 0.14$; (B) 0.3–0.6 m, $p_{NO_3\text{-}N} = 0.01$, $p_{NH_4\text{-}N} = 0.01$; (C) 0.6–0.9 m, $p_{NO_3\text{-}N} = 0.1$, $p_{NH_4\text{-}N} = 0.1$; (D) 0.9–1.2 m, $p_{NO_3\text{-}N} = 0.16$, $p_{NH_4\text{-}N} = 0.16$. One-factor analysis with Tukey's honestly significant difference (HSD). Means ± standard deviation. Lowercase letters a and b indicate the significance of NO_3-N (%) within the same sub-figure. Uppercase letters A and B indicate the significance of NH_4-N (%) within the same sub-figure. Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent, and 100 indicates a pure stand. AA, alfalfa pure stand; MF, meadow fescue; MF/AA, meadow fescue/alfalfa mixtures; RP, ribwort plantain pure stand; RP/AA, ribwort plantain/alfalfa mixtures.

of ribwort plantain on the soil was observed in both trial years, the yields and shares of mixture plants from all mixture species and the weather conditions in both trial years varied greatly. The outlined results show the tested mixture and pure stands of alfalfa, ribwort plantain, and meadow fescue under precipitation amounts that were below the long-term mean in 2019, as well as under precipitation amounts that deviated from the long-term mean in 2021.

4.1 | Ribwort plantain enhances forage accumulation

Ribwort plantain developed vigorously and thus contributed negatively to alfalfa growth (Tables 2 and 3). The ribwort plantain is a very competitive plant species that has moderate demands for site conditions (Ellenberg & Leuschner, 2010). A pot experiment with ribwort plantain showed that even under competition stress and lack of nutrient supply, it did not initiate reproduction and did not show higher costs of chemical defense; in contrast, the aboveground dry matter losses were less than under favorable environmental conditions (Marak et al., 2003). Ribwort plantain developed faster than alfalfa and meadow fescue in the cooler spring of 2021, while in the warmer spring of 2019, the development of both ribwort plantain and meadow fescue was roughly the same. According to Marak et al. (2003), this can be explained by the fact that ribwort plantain grows well under cool temperatures. Alfalfa is susceptible to light competition from weed plants during its development (Veronesi et al., 2010). Alfalfa is a drought-resistant plant that, similar to ribwort plantain (Pol et al., 2021), has a deep root system. However, the roots of young alfalfa plants are not yet sufficiently developed to withstand drought stress as they cannot reach deep soil layers and can be damaged (Prince et al., 2022; Sheaffer et al., 1988). Under dry conditions, N-uptake is inhibited in grasses due to drought stress, as the growth rate decreases (Burity et al., 1989). Similar cultivation methods for alfalfa in the literature have thus far achieved a higher FA in the first year of cultivation compared to the present trial with 2 t ha^{-1} in three cuts. Over three cuts, Rakotovololona et al. (2019) reached an alfalfa FA of 8.9 t ha^{-1} in the first growing year. Masoni et al. (2015) found an alfalfa yield of 15 t ha^{-1} over five cuts in the first growing year. Koch (1997) reported an

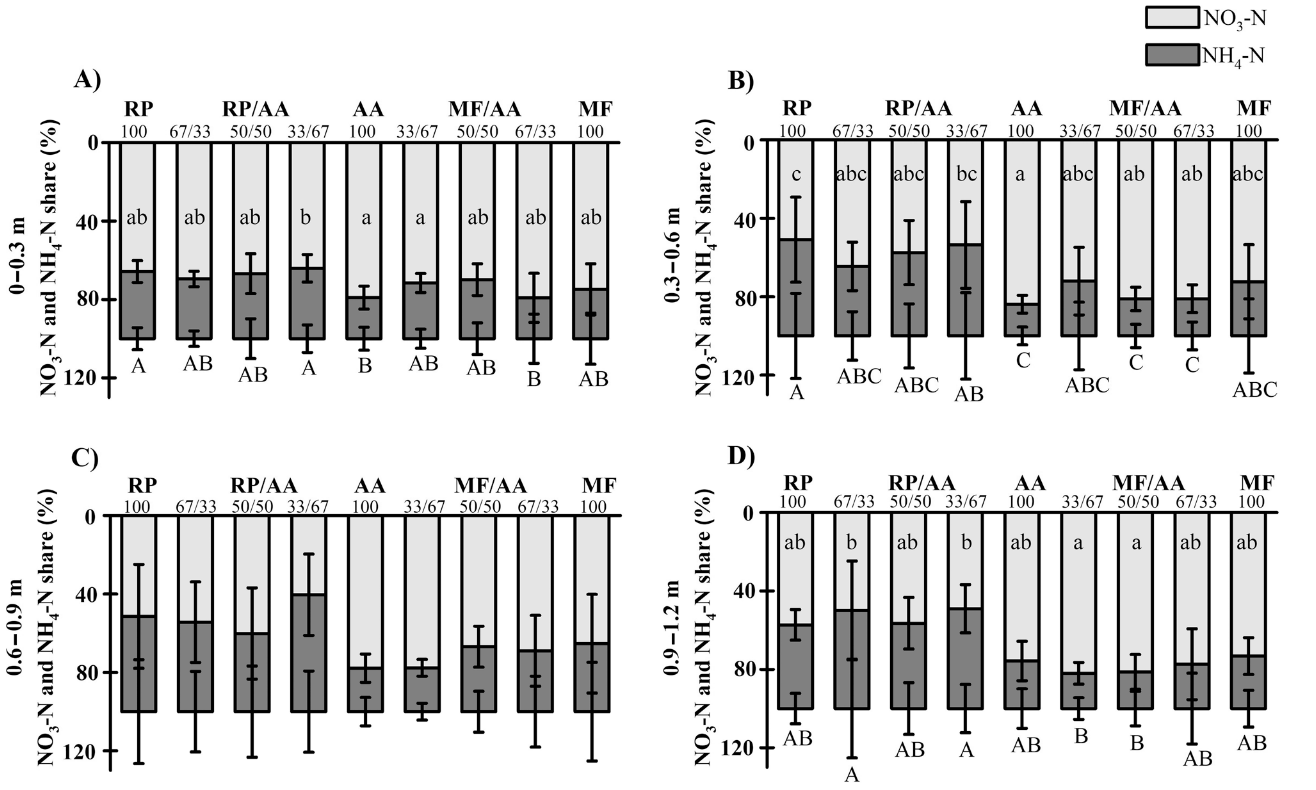

FIGURE 7 NO$_3$-N to NH$_4$-N (%) share in total N$_{min}$ at different soil depths in the 2021 field trial on September 14, 2021: (A) 0–0.3 m, $p_{NO_3\text{-}N} = 0.04$, $p_{NH_4\text{-}N} = 0.04$; (B) 0.3–0.6 m, $p_{NO_3\text{-}N} = 0.003$, $p_{NH_4\text{-}N} = 0.003$; (C) 0.6–0.9 m, $p_{NO_3\text{-}N} = 0.09$, $p_{NH_4\text{-}N} = 0.09$; and (D) 0.9–1.2 m, $p_{NO_3\text{-}N} = 0.001$, $p_{NH_4\text{-}N} = 0.001$. One-factor analysis with Tukey's honestly significant difference (HSD). Means ± standard deviation. Lowercase letters a and b indicate significance of NO$_3$-N (%) within the same sub-figure. Uppercase letters A and B indicate significance of NH$_4$-N (%) within the same sub-figure. Numbers 67/33, 50/50, and 33/67 indicate mixture proportions in percent, and 100 indicates a pure stand. AA, alfalfa pure stand; MF, meadow fescue; MF/AA, meadow fescue/alfalfa mixtures; RP, ribwort plantain pure stand; RP/AA, ribwort plantain/alfalfa mixtures.

8.8–11.3 t ha^{-1} dry matter yield on clayey silt. A practical test of the same alfalfa variety under similar soil and climatic conditions in Germany in 2021 showed FA of 2.5 t ha^{-1} over two cuts in annual cultivation, with very low values for the first cut, similar to the present trial (Kling & Bruckner, 2024).

The FA of the 67/33% and 50/50% ribwort plantain/alfalfa treatments were significantly higher at the second (2021) and the third cutting dates (2019, 2021) compared to the reference alfalfa/meadow fescue mixtures (Table 2). Skinner and Gustine (2002) found that the ribwort plantain yield was greater in September than in July, increasing by 62% in the control and 29% in a watered treatment. The imposition of summer drought on ribwort plantain increased winter survival from 3% in the wet treatment to 41% in the dry treatment (Skinner & Gustine, 2002). Rumball et al. (1997) also reported higher yields in autumn. However, most of the yield (>90%) was accounted for by the dry matter yield of ribwort plantain. The FA of ribwort plantain doubled over three cuts, both under dry conditions (2019) and higher precipitation (2021). Dembek et al. (2015) observed the effects of ribwort plantain on the yield and quality of sward in grasslands between 2006 and 2011 and found that ribwort plantain produced the highest grassland yields compared to the other species studied under dry weather conditions. In this study, ribwort plantain produced the highest FA in pure stand and with proportions between 84% and 97% in the mixture treatments with alfalfa.

The aucubin in ribwort plantain forage has an antimicrobial effect on ruminants. Ribwort plantain cuttings are well degradable in the rumen of ruminants and promote milk production (Mangwe et al., 2020). Ribwort plantain is suitable as an additive for sheep fodder because it significantly lowers the number of certain parasites in the feces (Reza et al., 2021). Furthermore, the urine of ribwort plantain-fed cattle reduces NO$_3$-N levels in soil and nitrous oxide emissions on pastures (Judson et al., 2019; Peterson et al., 2022; Simon et al., 2019).

The aucubin content varies in ribwort plantain depending on the time of harvest. Before flowering, the aucubin content is very low in every plant organ and reaches its maximum in autumn (European Medicines Agency, 2011). The new leaves of ribwort plantain contain more N and less aucubin (Bowers & Stamp, 1992). The aucubin content of leaves increases during the growing period of ribwort plantain but remains stable in the main growing period in the summer (Tamura & Nishibe, 2002). Darrow and Deane Bowers (1997) found a 0.5% aucubin content in leaf dry matter harvested in July and 4%–5% after harvests in September and October. Dietz et al. (2013) recorded 0.5%–1% aucubin in July, 5% in August, and 3%–4% in September.

Results from this work can inform organic farming practices, not only with regard to securing FA under dry conditions but also as a recommendation for preventive groundwater protection. The results obtained here also indicate a recommendation for ribwort plantain sowing density in mixtures with alfalfa due to the competitive growth pattern of ribwort plantain. A 25% or lower sowing density of ribwort plantain is recommended in a mixture with alfalfa. Depending on the 1000-grain mass of ribwort plantain seed, this leads to recommended seed rates of 0.75–1.0 kg ha^{-1} in the mixture. Based on our observations, if the sowing rate of ribwort plantain is reduced to about 25% and more than one forage legume species is added to the mixture, such as white clover (*Trifolium repens* L.) or red clover (*Trifolium pratense* L.), it can be assumed that the FA of the legumes in the mixtures will increase. Mixtures of more than two forage legume species showed higher yield performance (Kirwan et al., 2007), as the legume dry matter proportion in the total mixture yield should be between 30% and 40% for high quality forage for ruminants (Broderick, 1995; Nußbaum, 2007). A further recommendation is to sow ribwort plantain to a depth of 0.5–1 cm (loamy soils) or 1–1.5 cm (sandy soils) due to its small seed size. Furthermore, the cutting time of the ribwort plantain must not be too late, as it tends to form seeds quickly, which could become problematic in the subsequent organic crop rotation. As both alfalfa and ribwort plantain can use water reserves in the lower soil layers due to their deep roots (Pol et al., 2021; Sheaffer et al., 1988), caution should be taken in water protection areas to ensure that groundwater formation is not impaired. Further research is needed in this area. In this regard, a comparison of the yield performance of different ribwort plantain varieties under field conditions would be particularly useful for organic farming.

4.2 | NO_3-N shifts to NH_4-N with ribwort plantain

Although the ribwort plantain/alfalfa mixtures were very heavily weeded in the first trial, the presence of alfalfa even in small quantities, affected the plant-available N in its pure strand and in the treatments with meadow fescue. The results of the NO_3-N and NH_4-N ratio in autumn showed more NO_3-N under 100% alfalfa, which agreed with the findings of Dollete et al. (2024) and Wery et al. (1986), as dry conditions do not inhibit the nitrification of N. The symbiotic N fixation of alfalfa depends on water availability in the soil (Dollete et al., 2024; Wery et al., 1986). However, the mechanisms by which alfalfa adapts to environmental stress, in particular to drought, are still not well known (Soba et al., 2019). The root nodules in alfalfa roots go through anaerobiosis during drought periods with high water stress, which decreases oxygen uptake in the soil and affects the nodules (Becana et al., 1986). Dollete et al. (2024) showed that drought conditions led to lower biomass and reduced symbiotic N fixation in alfalfa in a glasshouse experiment. By damaging roots and nodules, they may release organic N compounds, which are converted into NH_4-N and subsequently into NO_3-N through nitrification (Dollete et al., 2024). Other studies have concluded that drought periods lead to a boost in the alfalfa yield (Athar & Johnson, 1996). High levels of environmental stress can lead to subsequent nodule growth in forage legumes as an evolutionary attempt to sustain or compensate for the efficiency of the plant (Bordeleau & Prévost, 1994). Alfalfa roots usually expand their growth (width and depth) in soil during drought periods and increase their N concentration (Antolín et al., 1995; Soba et al., 2019). The first differences in the plant-available N composition in the shares of NO_3-N and NH_4-N were observed at the first cutting date at the end of June 2019. These results can be explained by the weather conditions in 2019, as the time from sowing to the first cutting date was 1 month longer compared to 2021. Differences were observed in June 2019, as the aucubin content in ribwort plantain was likely higher than in May 2021. The highest aucubin concentration is found in intermediate-aged leaves and the lowest in mature leaves of ribwort plantain (Bowers & Stamp, 1992). The experiments in this study were carried out under generally unfavorable soil and climate conditions with a high ribwort plantain sowing rate, whereby alfalfa was heavily displaced by ribwort plantain. According to Burity et al. (1989), up to 50% of the total N in grass in alfalfa mixtures could be derived from N fixation. With a higher share of alfalfa in a mixture, fixing more N, the nitrification inhibition of ribwort plantain was more pronounced in soil.

In October (Figures 6 and 7), a clearer NO_3-N shift to NH_4-N was observed at 0.3- to 1.2-m soil depth with ribwort plantain/alfalfa mixtures, as influenced by the presence of ribwort plantain. The relationship between the initially sown proportion of ribwort plantain seeds, the ribwort plantain growth, and the residual NO_3-N reserves in the soil was not clearly recognizable. Ribwort plantain did not go to seed during 2019 and 2021, but lower seed proportions tended to accumulate more forage in mixtures. Ribwort plantain had generally higher FA with low NH_4-N shares in 50/50% alfalfa treatment in autumn in 2019 and 2021 (Table 2; Figures 6 and 7).

In our field trials, we used a ribwort plantain cultivar that generally has a high aucubin content. However, there are other ribwort plantain cultivars that may not have the same effects under different soil types and drier climate conditions. Pol et al. (2024) showed that yield performance and aucubin content vary among commercial cultivars. Furthermore, Pol et al. (2024) found that the aucubin content varies under different light, soil, and water conditions.

5 | CONCLUSION

We showed that ribwort plantain has the overall potential to contribute to nitrate reduction and yield enhancement. Despite the significant annual differences, the same effect of an overall nitrate reduction was observed. We had a site with challenging conditions for agriculture in a water-protection area with strict rules for agricultural management. Therefore, based on our results, we conclude that ribwort plantain has a nitrate-reducing effect. We would expect the same effect, weaker or more pronounced, to occur in a new field trial with better soil conditions. However, it must also be emphasized that the influences of the different weather conditions should be more thoroughly investigated under different soil conditions.

FA was highest, with the presence of ribwort plantain, both in pure stands and mixtures. Ribwort plantain outperformed alfalfa with a share of >80% in all mixture treatments, with a tendency to increase FA by early autumn. For this reason, it is advisable for practitioners to greatly reduce the proportion of ribwort plantain seed and maintain it below 25% of its pure seed rate in mixtures with alfalfa or other forage legumes. A combination of ribwort plantain with, for example, two forage legume species would be conceivable in practice under dry conditions to increase the share of legumes in the mixture. In both field trials, the meadow fescue/alfalfa mixtures resulted in lower FA for meadow fescue compared to pure stands. However, due to the limitations of the study, it should be emphasized that the effects presented might not be the same under, for example, clay soils or even drier locations.

In autumn, the NO_3-N content at 0–0.3 and 0.3–0.6 m was higher in the treatments with 100% alfalfa and meadow fescue and their mixtures compared to ribwort plantain. The ratio gap continued to increase from 0.6 to 0.9 m, as ribwort plantain clearly affected the NO_3-N share, as all treatments with it had higher NH_4-N shares.

As a new crop rotation element, ribwort plantain can directly promote the resource efficiency of an agroecosystem, as the cultivation is based solely on the utilization of natural resources and their interactions. Furthermore, the introduction of ribwort plantain to agroecological systems can help farmers cope with the adverse effects of climate change. This study can also help farmers in water protection areas to reduce the risk of agricultural cultivation restrictions for forage legumes. Additionally, with meadow fescue as a less popular grass species, the study expanded the number of plant species on agricultural land.

AUTHOR CONTRIBUTIONS

Tsvetelina Krachunova: Conceptualization; formal analysis; investigation; methodology; project administration; visualization; writing—original draft. **Sonoko Bellingrath-Kimura**: Supervision; validation; writing—review and editing. **Knut Schmidtke**: Conceptualization; funding acquisition; methodology; project administration; supervision; validation; writing—review and editing.

ACKNOWLEDGMENTS

The authors thank Hannah Scharfstädt and Martin Scholz from University of Applied Sciences Dresden, who were involved in the field work, plant and soil sampling, and sorting in 2019. The authors would also like to thank Wassergut Canitz GmbH of public utility Leipzig for providing the test areas and Ms. Caroline Golatowski and Dr. Bernhard Wagner for their active support of the research project.

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original data that support the findings of this original research manuscript are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Krachunova, T., Bellingrath-Kimura, S., & Schmidtke, K. (2025). Annual ribwort plantain and alfalfa mixtures enhance forage accumulation and reduce nitrate. *Agronomy Journal*, *117*, e70098. https://doi.org/10.1002/agj2.70098