Grass-legume mixtures can reduce nitrogen fertiliser requirement: a three-year, eight-site study

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Abstract

We investigated whether grass-legume mixtures can reduce the need for nitrogen (N) fertiliser while maintaining yield. We analysed data from an eight-site and three-year experiment where species diversity (the sown richness and proportions of species), seeding density and N fertiliser regime were manipulated. At each site, the experimental design included fifteen community types: four monocultures and eleven four-species mixtures, which systematically varied the sown proportions of two grasses and two legumes. These communities were sown at two densities and at two N fertiliser levels. Equi-proportional grass-legume mixtures with low N input consistently produced comparable yield to the best-performing grass monoculture with regular N input. Thus, compared to the best-performing grass monoculture, grass-legume systems can reduce N fertiliser requirements while sustaining yield. Beneficial interactions between the two functional groups contributed to this effect, potentially driven by the ability of legume species to symbiotically fix N_2. However, the magnitude of these benefits varied across sites. These insights support the design of optimal mixtures under lower N supply, highlighting their potential to enhance N use efficiency and promote more sustainable agricultural practices without the need for specific environmental or management conditions.

Keywords: grasslands, multi-site analysis, multi-species mixtures, nitrogen fertiliser

Introduction

Nitrogen (N) fertiliser is widely used for enhancing forage production, but poses environmental challenges, including greenhouse gas emissions as well as ecosystem eutrophication. To address these issues while maintaining yield, sustainable alternatives are needed. Grass-legume mixtures can exploit symbiotic N_2 fixation and enhance soil fertility under low level of N application, often outperforming grass monocultures in yield (Finn *et al.*, 2013). Although previous studies have explored the effects of species diversity on N fertiliser use (Nyfeler *et al.*, 2009), they have been limited to single-site experiments. Thus, there is a lack of understanding of whether these effects are consistent across different locations. This study addresses this gap by providing, for the first time, a multi-site and multi-year analysis investigating the joint effect of manipulating species diversity and N fertiliser application.

Materials and methods

Field experiments were conducted over three years and at eight sites that spanned a range of European climate types. The core design included 15 communities: four monocultures (one for each species) and 11 mixtures of the four functional groups: fast- and slow-establishing grass, and fast- and slow-establishing legume. The species representing each group varied by site and included *Lolium perenne* (fast), *Dactylis glomerata* (slow), *Phleum pratense* (slow), *Trifolium pratense* (fast), *Medicago* *sativa* (slow), *Trifolium repens*, and *Trifolium ambiguum* (slow). Each community was sown at two overall seeding densities (low and high) for the same species compositions. Depending on the site, either all or most community by density combinations were sown under two N fertiliser treatments (low N and regular N), leading to between 48 and 60 plots at each of seven sites, while one site expanded to 100 plots by adding extra communities. The amount of N applied under the two treatments was adapted to local conditions and so varied across sites: the low N treatment ranged from 0 to 100 kg/ha per year (on average 39.4 kg/ha per year), while the regular N treatment ranged from 120 to 240 kg/ha per year (on average 170 kg/ha per year). Total biomass yield was measured annually in each plot. This study was part of the Agrodiversity Experiment; while the data analysed here from multiple N levels is publicly available in Kirwan *et al.* (2014), there has been no prior analysis published that jointly analysed the effects of species diversity and N manipulation (although one of the sites has previously published their results, see Nyfeler *et al.* (2009)). To analyse the effects of species diversity and N fertilisation on yield, Diversity-Interactions models (Kirwan *et al.*, 2009; Connolly *et al.*, 2013; Moral *et al.*, 2023) were used within a mixed-effects modelling framework to account for variability from site to site. Analysis of the data was conducted in R with the nlme package (Pinheiro *et al.*, 2025). Differences between two communities were tested using Wald tests.

Results and discussion

Across the eight sites and the three years, under the low N treatment, monocultures yielded on average 7.59 t/ha, the best-performing grass monoculture (the slow-establishing grass) yielded 6.77 t/ha, while the equi-proportional mixture (25% of each species at sowing) yielded 11.32 t/ha (Figure 1). Under the regular N treatment, monocultures yielded 9.57 t/ha on average and the best grass monoculture yielded 10.38 t/ha, compared to 12.41 t/ha for the equi-proportional mixture. In the three individual years, on average across sites, the equi-proportional mixture with low N yielded 10.87, 11.35 and 11.74 t/ha, while monocultures with regular N on average yielded 9.26, 9.75 and 9.69 t/ha, respectively; the best-performing grass monoculture with regular N yielded 9.5, 11.22, and 10.41 t/ha (Figure 1). However, variations in mixture performances across sites and years were evident (see also Finn *et al.* (2013)), likely due to fluctuations in precipitation and temperature that influenced plant growth and N uptake (Suter *et al.*, 2015).

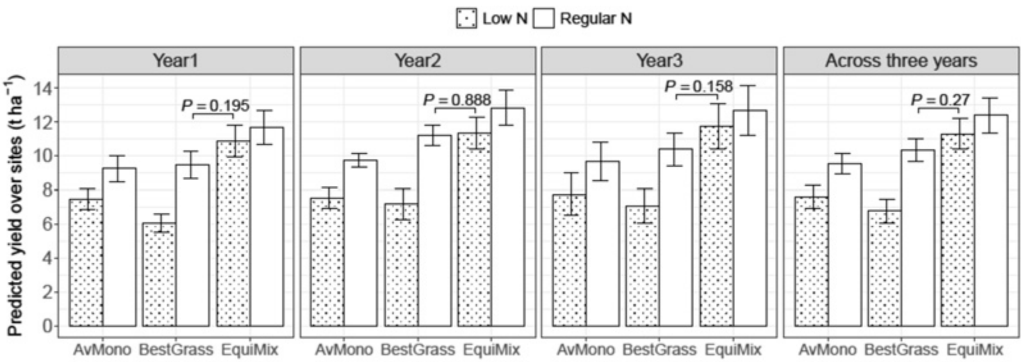

Figure 1. Predicted yield (±SE) of averaged monocultures over four species (AvMono), the best-performing grass monoculture (BestGrass), and the equi-proportional mixture (EquiMix) across sites for each year and averaged across the three years, under the two N fertiliser treatments. The p-value (*P*) shown in each panel tests for a difference in predicted yield between the best grass monoculture under regular N level and the equi-proportional mixture under low N level.

On average across sites, the estimated effects of species diversity on biomass yield differed between N fertiliser levels and in all the three years. Additionally, synergistic interactions between grass and legume species were detected in each of the three years, highlighting the benefits of grass-legume mixtures for grassland yields. Furthermore, yields of the equi-proportional mixtures at low N application was not significantly different from those of the best-performing grass monoculture at regular N level, both in each year and on average across the three years (Figure 1). This suggests that grass-legume mixtures with lower N application can match the yields of the highest-yielding grass monocultures with higher N application. The benefits of grass-legume mixtures across all sites and both fertiliser levels suggest that these mixtures are widely effective and do not require specific environmental or management conditions for successful implementation.

Conclusion

This study presents a novel multi-site, multi-year analysis of data from the Agrodiversity experiment to assess the joint impacts of grass-legume mixtures and N fertiliser manipulation on yield. Across eight sites over three years under two N fertiliser levels (with an averaged difference of 130 kg/ha per year between the two N levels), grass-legume mixtures under low N level achieved yields comparable to the best-performing grass monoculture (the slow-establishing grass) under the regular N level. Multi-site analyses provide a strong basis for generalising recommendations.

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