Grass-clover leys for a sustainable N yield: *Trifolium pratense* cultivar × mixture effects

Gamper H.A.[1], Mairhofer F.[2], Ceccon C.[1], Matteazzi A.[2], Gauly M.[1] and Peratoner G.[2]
[1]*Free University of Bolzano, Faculty of Science and Technology, Piazza Università 5, 39100 Bolzano/Bozen, Italy;* [2]*Laimburg Research Centre, Research Area Mountain Agriculture, Laimburg 6, Vadena/Pfatten, 39040 Ora/Auer, Italy*

Abstract

Grass-clover leys allow sustainable intensive forage and livestock production but large pedoclimatic differences make mixture × cultivar trials necessary to identify site-specific optimal combinations. We addressed experimentally the questions: (1) whether cultivars of *Trifolium pratense* must be evaluated in a specific seed mixture, or whether their biomass and N yield and symbiotic N_2 fixation can be ranked in any mixture; and (2) whether mixtures perform differently depending on the choice of *T. pratense* cultivar. The trial was set up with three cultivars (Milvus, Semperina, Spurt) and three regionally recommended grass-clover mixtures (IR, KG, WW), differing, among others, in *T. pratense* abundance. At the third cut after autumn seeding, *T. pratense* dominated the dry matter and N yield in mixture- and cultivar-specific manners. Irrespective of mixture and *T. pratense* cultivar, 56.7±1.1% of total N was acquired via symbiotic N_2 fixation, owing to compensatory dynamics in sward structure and degree of reliance on N_2 fixation. Cultivars Spurt and Milvus boosted dry matter and N yield, with cv. Spurt accumulating most N, and cv. Semperina tending to acquire proportionally most N via symbiosis. Our findings suggest that mixture × cultivar evaluation trials may not always be necessary as long as the mixtures are of similar composition and demonstrate N yield stability, despite different structure.

Keywords: agronomic performance, cultivar, leys, symbiotic N_2 fixation, red clover

Introduction

Multispecies temporary grassland for intensive forage production regenerates soil fertility, thereby contributing to globally sustainable food production through reliance on local feed stuff (Jan *et al.*, 1992). In climatic regions with cold winters and thus relatively short growing seasons, but sufficient precipitation, *Trifolium pratense* is the forage legume of choice to increase forage quality and late-season yield. However, despite big strides in breeding and designing grass-clover mixtures (Grieder *et al.*, 2019), it is still unclear: (1) whether the relative performance of cultivars of *T. pratense* must be tested in specific target mixtures or if the ranking of cultivar performance in one standard mixture is also meaningful for other mixtures; and (2) whether cultivars of *T. pratense* exert consistent effects on parameters of agronomic interest across different mixtures. We addressed these questions in respect to the agronomic traits of dry matter (DM) and N yield, and the sustainability trait of symbiotic N_2-fixation, using a two-factorial seed mixture and cultivar evaluation trial.

Materials and methods

Three cultivars of *T. pratense* (Milvus, Semperina, Spurt), which had been found to perform similarly in a monospecific cultivar trial at the same site, were alternatively used in three grass-clover seed mixtures for leys [KG and WW, recommended for South Tyrol, and IR, recommended for Austria (Krautzer *et al.*, 2020)], differing primarily in seed weight proportion of several species and showing an approximate two-fold reduction in the abundance of *T. pratense* from 27.3% in KG to 13.3% in IR and to 5.0% in WW. The other legume components in the mixtures were *Trifolium repens*: IR, 8.8%; KG, 4.6%; WW, 8.0%, and *Trifolium hybridum*: KG, 4.5%; missing in the other seed mixtures. The trial was setup on 29.08.2019 in Teodone/Dietenheim (South Tyrol, NE Italy; 46° 48' 8.064' N, 11° 57' 23.908' E; 891 m a.s.l., 8.4 °C mean annual temperature, 733.5 mm mean annual precipitation) in a randomized complete block design with three replicates and a plot size of 1.2×7.2 m. The total soil N content was 1.17 g kg^{-1} prior to application of 40 kg N ha^{-1} and 53 kg ha^{-1} K in mineral form in spring and of 15 m^3 ha^{-1} digested cow slurry after the first and second of four annual harvests. The yield of *T. pratense*, *T. repens*, and of the remaining fraction (mostly grass) of the third harvest on 12 August 2020 was assessed by means of manual species separation from a 0.25 m^2 sampling area per plot. The N concentrations ([N]) and $\delta^{15}N$ signatures of the shoots were determined by isotope ratio mass spectrometry. Dry matter (DM) yield was assessed at plot level, as was the [N], measured according to Dumas. The proportion of N derived from the atmosphere (pNdfa) by symbiotic N_2 fixation was calculated at plot level, using ^{15}N natural abundances and the formula $(\delta^{15}N_{Grasses} - \delta^{15}N_{Clover\ species}) / (\delta^{15}N_{Grasses} - B)$, where $\delta^{15}N$ stands for the $^{15}N/^{14}N$ isotope ratio in per mil, following Unkovich *et al.* (2008). The B-values used were the means for shoots of the *Trifolium* spp. determined of Carlsson *et al.* (2006). N yields, Ndfa, and N derived from soil (Ndfs) were calculated as the products of the DM yields and [N], pNdfa, and (1-pNdfa), respectively. The data was analysed by ANOVA accounting for seed mixture, *T. pratense* cultivar, their interaction, and block, all treated as fixed factors, followed by LSD test. N yield and pNdfa of *T. repens* were square root-transformed to meet the ANOVA assumptions. The significance level was set at P=0.05.

Results and discussion

The DM and N yields of the swards were similarly affected by seed mixture and *T. pratense* cultivar, but the effect of seed mixture was about four-fold stronger than that of cultivar for the yield of *T. pratense* and even ten-fold stronger for that of *T. repens* (Figure 1) due to the differences in the abundance of the individual clover species. For none of the investigated parameters was there an interaction found between seed mixture and *T. pratense* cultivar. The DM yield of the seed mixtures ranked: KG > WW with IR not significantly differing from each of them, and the DM and N yields of the *T. pratense* cultivars: Spurt > Semperina with Milvus not significantly differing from each of them. Ndfa contributed on average 56.7±1.1% (n=27, standard error of the mean) to the N yield of the swards, irrespective of seed mixture, *T. pratense* cultivar, and mixture × cultivar combination. Cultivar Semperina tended to acquire most N via symbiosis, but this positive trait traded off against a low DM yield in mixture. The *T. repens* and grass fraction of the seed mixtures largely compensated *T. pratense* abundance and cultivar effects on pNdfa. Compensatory dynamics between mixture components hence explain the stable and high N yields of the evaluated grass-clover ley mixtures as known from other studies (Finn *et al.*, 2013; Hejduk *et al.*, 2010). However, the agronomic relevance of these findings from just one harvest in just the first year of three cultivation years needs to be verified with the data of the entire three cultivation years and will afterwards have to be confirmed in multi-site trials under different fertilization regimes.

Conclusions

Absence of statistical interactions between the factors seed mixture and *T. pratense* cultivar for all agronomically relevant parameters of the third harvest in the first cultivation year suggest that testing cultivars in all possible target mixtures might not always be necessary. Our findings show, however, that testing the cultivars in mixed swards is necessary to identify the best performing *T. pratense* cultivar.

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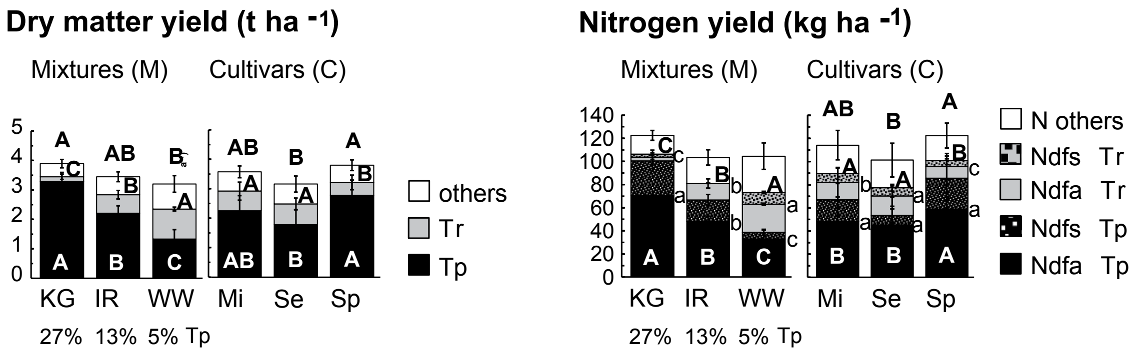

ANOVA:	M($F_{2,16}$):	C($F_{2,16}$):	M x C($F_{4,16}$):
Sward:	4.768*	4.095*	0.488^{ns}
Tr:	40.720***	4.791*	1.285^{ns}
Tp:	28.847***	7.568**	0.231^{ns}

Note: Block($F_{2,16}$) was never significant.
*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$

ANOVA:	M($F_{2,16}$):	C($F_{2,16}$):	M x C($F_{4,16}$):
N sward:	3.461^{ns}	4.762*	1.664^{ns}
N Tr:	37.550***	4.031*	1.101^{ns}
Ndfa Tr:	60.912***	12.029***	1.200^{ns}
N Tp:	29.238***	8.051**	0.169^{ns}
Ndfa Tp:	11.060***	0.598^{ns}	0.703^{ns}

Note: Block($F_{2,16}$) was never significant.
*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$

Figure 1. Differences in dry matter and nitrogen (N) yield of three grass-clover seed mixtures (KG, IR, WW), differing in seed weight percentage and cultivar identity [Milvus (Mi), Semperina (Se), Spurt (Sp)] of *Trifolium pratense* (Tp). Means, standard errors, and associated statistical results of ANOVA and multiple comparisons by LSD are shown for the entire sward and the *T. pratense* and *T. repens* (Tr) components (n=9) in capital letters and for the amounts of N derived from the atmosphere (Ndfa) and soil and fertilizer (Ndfs) by the two *Trifolium* species in small letters.

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