Including forbs in multi-species leys did not affect soil organic carbon and nitrogen stocks

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Abstract

Inclusion of forbs into grassland mixtures is expected to have multiple benefits in animal nutrition, especially if those forbs are rich in secondary plant metabolites. Increased functional group diversity and the presence of deep-rooting forb species, in particular, may also increase soil carbon sequestration in temporary grasslands. In April 2020, we established four temporary grassland mixtures in Frick, Switzerland: grasses, grasses with legumes, grasses with plants rich in essential oils, and grasses with tannin-rich plants. Each mixture was replicated in four randomised plots that were grazed by dairy cows. In March 2020 and February 2024, soil cylinders were taken in the 0–10, 10–20 and 30–50 cm soil layers. Bulk density, soil organic carbon (SOC) and total soil nitrogen (STN) concentrations were measured for stock calculation. Across mixtures, SOC and STN stocks increased from 2020 to 2024 in the 0–10-cm layer as a consequence of increased soil density. Mixture affected neither SOC nor STN stocks or concentrations, but had a minor effect on bulk density in the 10–20-cm layer. The results show that an increase in functional group diversity may not always affect grassland carbon sequestration.

Keywords: multi-species swards, soil organic carbon, temporary grassland

Introduction

Including forbs into temporary grassland mixtures can provide multiple benefits in animal nutrition, especially if those forbs are rich in secondary plant metabolites (Cooledge *et al.*, 2022). Increased functional group diversity and the presence of deep-rooting forb species may also increase other ecosystem functions of temporary grasslands, like sequestration of soil organic carbon (SOC). While root mass and carbon input into soils have been shown to be greater in temporary grasslands containing herbs compared to grass and grass-legume mixtures, their effect on SOC sequestration has seldom been studied (Cooledge *et al.*, 2022).

Accordingly, we assessed the change of SOC and soil total nitrogen (STN) stocks of four temporary grassland mixtures over four years, expecting greater increases in SOC and STN stocks in the two mixtures containing forbs than in the grass and grass-legume mixtures.

Materials and methods

In April 2020 we established four temporary grassland mixtures in Frick, Switzerland, on an organically managed arable field which had previously been part of an arable-ley rotation, and that was ploughed prior to seeding. All seed mixtures contained the grass species *Poa pratensis*, *Lolium perenne*, *Festuca rubra* and *F. arundinacea*. The grass mixture (G) contained only these species; the grass-legume mixture (L) additionally *Trifolium pratense*, *T. repens* and *Medicago sativa*; the tannin-rich mixture (T) *Lotus corniculatus*, *Sanguisorba minor*, *Cichorium intybus* and *Plantago lanceolata*; the essential-oil mixture

(EO) *Achillea millefolia*, *Artemisia absinthium*, *Carum carvi*, *Origanum vulgare* and *Thymus pulegoides*. Each mixture was replicated in four plots, with two subplots per plot, from which biomass was sampled following two different sampling schemes: immediately before stocking, or additionally in the middle of the resting period. The whole area was grazed by dairy cows with an average stocking density of 18 livestock units per hectare and five stocking periods per year. In September 2021, all mixtures received 150 kg ha^{-1} total nitrogen in the form of slurry. Mixture G was additionally fertilised in spring 2022 and 2023 with 80 kg ha^{-1} y^{-1} nitrogen in the form of commercial fertiliser with 12% organic nitrogen concentration.

In March 2020, before ploughing, and in February 2024, soil cylinders (98.17 cm^3) were taken in the 0–10, 10–20 and 30–50 cm soil layer at two GPS-positioned pseudoreplicates per subplot. Soil drybulk density (BD) was assessed by weighing the cylinders after drying at 40°C immediately after sampling. Total carbon (TC) and STN concentrations of both years were analysed in the same batch in 2024 by dry combustion (vario Max cube, Elementar, 900°C, 80 s, 80 ml O_2 min^{-1}) and a soil aliquot dried at 105°C for dry matter analysis. SOC concentration was calculated by subtracting the inorganic carbon concentration (500°C ignition loss for 5 h and subsequent dry combustion without O_2 at 900°C, 80 s) from TC. SOC and STN stocks were calculated by SOC/STN stock (Mg ha^{-1}) = (SOC/STN (g kg^{-1})*BD (g cm^{-3})*height of soil layer (cm))/10.

For each soil layer, mixed effects models with block, year, mixture, sampling scheme and their interactions as fixed effects, and subplot nested in plot as random effects, were fitted to model each dependent variable. In each case, a minimum adequate model was identified with the Akaike Information Criterion corrected for small sample size, retaining the block effect.

Results and discussion

In most cases, the minimum adequate model only contained the year effect (Table 1). In all soil layers, SOC and STN concentrations decreased between the years (on average 0.63 g kg^{-1} SOC in 0–10 cm, 1.82 g kg^{-1} SOC in 10–20 cm and 0.47 g kg^{-1} SOC in 30–50 cm; Table 2). The slight decline may have been caused by ploughing of the field before the establishment of the mixtures as well as yearly variations in SOC/STN levels.

The SOC concentration was similar to other Swiss lowland sites with a high soil clay content of around 40% and a crop rotation including grass-clover leys (Gubler et al. 2019). Bulk density increased during the years, most likely due to the lack of tillage operations and the grazing activity of the cows, as commonly observed for grassland leys in arable rotations (Cooledge *et al.*, 2022). The increase of SOC and STN stocks from 2020 to 2024 in the 0–10-cm soil layer hence was due to the soil compaction effect.

Table 1. Significance (*p* values) of the effect of sampling year on soil organic carbon (SOC) and total soil nitrogen (N) stocks and contents as well as soil bulk density.

Soil layer	SOC stocks	N stocks	SOC content	N content	Bulk density
Upper	<0.0001	0.0154	0.0037	<0.0001	<0.0001
Middle	—	<0.0001	<0.0001	<0.0001	<0.0001
Lower	—	0.2885	0.0929	0.1230	0.0073

Dash, year effect not retained in minimum adequate model.

Table 2. Mean values (SD) of soil organic carbon (SOC) and total soil nitrogen (N) stocks and contents as well as soil bulk density in 2020 and 2024, averaged across mixtures.

Soil layer	Year	SOC stocks (Mg ha^{-1})	N stocks (Mg ha^{-1})	SOC content (g kg^{-1})	N content (g kg^{-1})	Bulk density (g cm^{-3})
0–10 cm	2020	36.46 (2.91)	3.72 (0.28)	29.2 (2.2)	2.98 (0.21)	1.25 (0.058)
	2024	38.75 (1.94)	3.80 (0.17)	28.6 (1.9)	2.80 (0.16)	1.36 (0.052)
10–20 cm	2020	36.15 (3.15)	3.69 (0.28)	28.7 (2.4)	2.93 (0.22)	1.26 (0.090)
	2024	35.53 (2.49)	3.54 (0.20)	26.9 (2.1)	2.68 (0.16)	1.32 (0.047)
30–50 cm	2020	38.27 (4.93)	4.05 (0.43)	14.4 (1.8)	1.53 (0.21)	1.33 (0.069)
	2024	37.47 (4.46)	3.98 (0.56)	13.9 (2.2)	1.48 (0.27)	1.36 (0.065)

The effect of the sward mixtures was only retained in the model predicting bulk density in the 10–20-cm soil layer ($p = 0.068$), with a trend for a higher bulk density in mixture G compared to the other mixtures. The absence of differences in SOC between sward mixtures and the overall decline in SOC and STN concentrations after four years show that there is no clear effect of sward mixture composition on SOC stocks. This is confirmed by the contrasting results obtained in the few studies investigating the development of SOC stocks under temporary grasslands differing in species composition. For example, 4-species mixtures had higher topsoil SOC concentrations compared to 16-species mixtures at nine years after establishment of an experimental grassland on former arable land (Mellado-Vázquez et al., 2016). By contrast, Cooledge *et al.* (2024) found increased soil organic matter concentrations after two years under both a grass-clover and a herbal ley, yet without differences between the two sward types. While many studies have found an increase in root mass and carbon inputs into soil in complex mixtures compared to simple mixtures (e.g. McNally *et al.*, 2015; Mortensen *et al.*, 2025), these effects may not necessarily translate into SOC sequestration, at least not during the time span that temporary grasslands usually occupy in a crop rotation. Differences in rooting depth might, however, have contributed to the trend for greater soil bulk density of mixture G at 10–20 cm depth compared to the other mixtures.

Conclusions

While including forbs into grass and grass-clover leys has previously been shown to increase root biomass, this study demonstrates that it does not necessarily affect SOC stocks over a period of four years, a typical duration for grassland leys. However, the effect of multi-species leys on SOC stocks should also be assessed cumulatively over several crop rotations.

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