

Supplemental material

Title: Forage species identity shapes soil biota in a temperate agroecosystem

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Supplemental material 1. Annual forage yield and species composition

Methods

Forage yield was estimated by destructive harvest of two 0.25 m² quadrats per plot prior to each of three hay cuttings. At each harvest, crop and weed biomass were segregated and forage biomass further sorted by species. Samples were dried at 60°C and weighed. Annual yield is the sum of estimated biomass at each cutting. Analysis of variance (ANOVA) was performed to identify yield differences across treatments using a mixed model with a fixed treatment effect and random block effect. Data were analyzed separately by year due to a significant treatment by year interaction. All analyses were performed in R statistical software (R Development Core Team, 2019).

Results

Supplemental Table S1. Annual dry matter yield from perennial forages in the second (2018) and third (2019) growing seasons following establishment in southeast Pennsylvania, USA. Values are the sum of dry matter (kg ha⁻¹ ± 1 standard error) from three hay cuttings each year averaged within treatment.

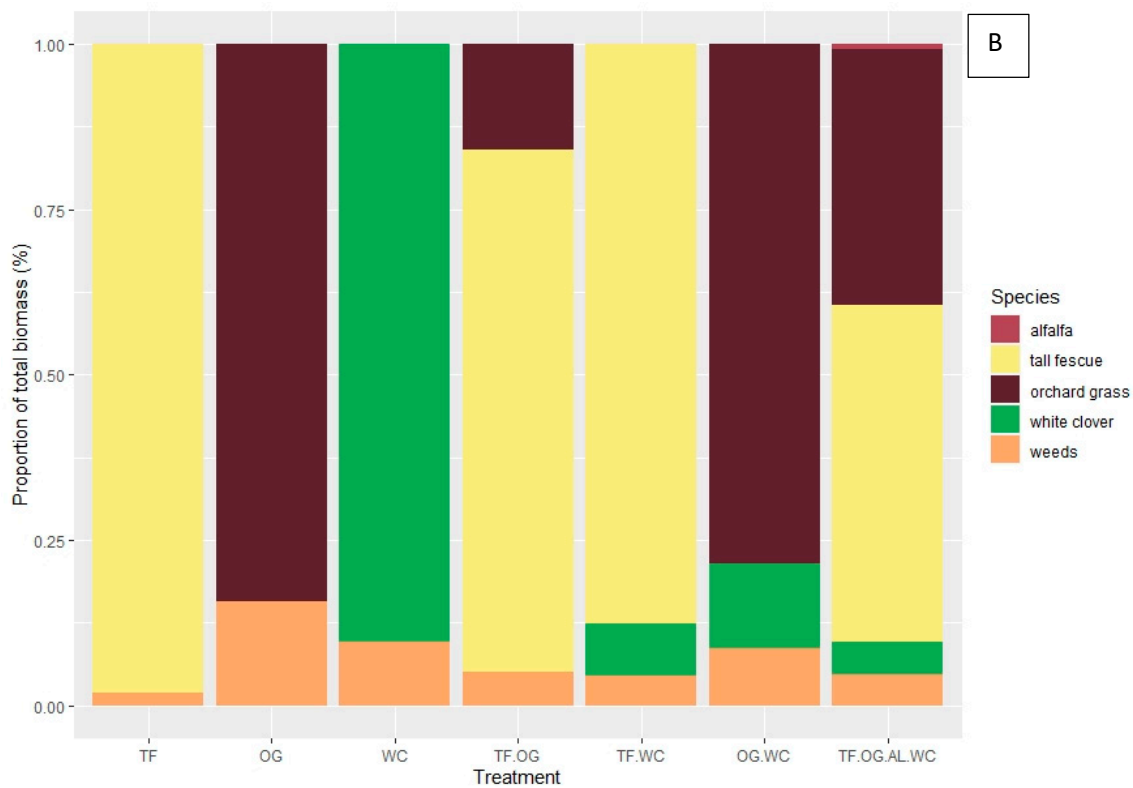
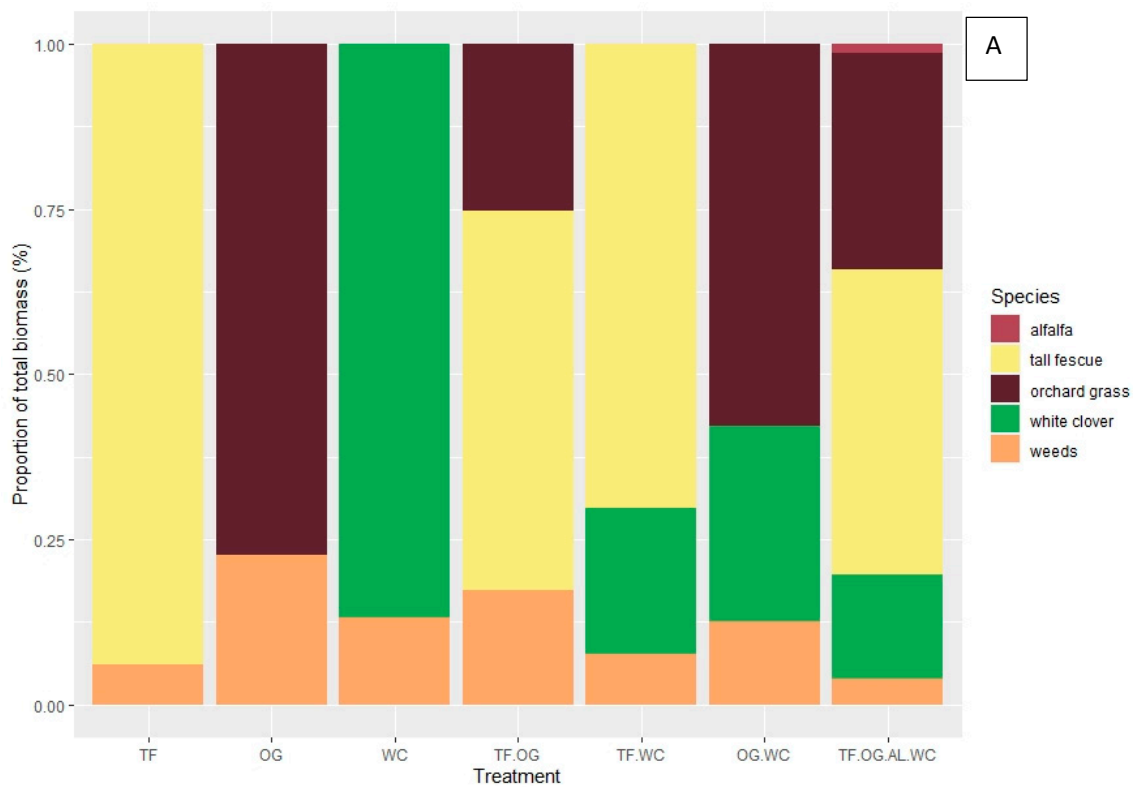
<u>Treatment</u>	<u>Annual dry matter yield (kg ha⁻¹)</u>	
	<u>2018</u>	<u>2019</u>
Tall fescue (TF)	5861.6±152.2 cd†	7603.6±480.0 ab
Orchardgrass (OG)	4130.1±530.8 d	5299.1±542.9 bc
White clover (WC)	5889.3±474.4 bcd	3739.4±7006. c
TF + OG	5297.4±972.8 cd	6652.6±779.7 ab

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TF + WC	8223.3 \pm 849.1 ab	9102.8 \pm 644.0 a
OG + WC	6805.0 \pm 540.2 bc	6750.6 \pm 606.3 ab
TF + OG + WC	8597.0 \pm 756.7 a	8110.4 \pm 1302.9 a

†Lowercase letters indicate differences among values within each column based on mean separation using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$.

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Supplemental Figure S1. Species composition in seven perennial forage cropping treatments at the time of spring cutting in the second (2018, A) and third (2019, B) growing seasons following establishment in southeast Pennsylvania, USA. TF, tall fescue; OG, orchard grass; WC, white clover.

Supplemental material 2. Soil inorganic nitrogen

Methods

Soil inorganic nitrogen (N) concentration (the sum of nitrate-N [$\text{NO}_3\text{-N}$] and ammonium-N [$\text{NH}_4\text{-N}$]) was determined on soil samples collected in for microbial and chemical analyses June 2018 and 2019 and represents available N at the time of first cutting each growing season. Extractable inorganic N was quantified on a 10 g (rock-free fresh weight) subsample extracted with 50 mL 0.5M K_2SO_4 and 1 hr of shaking followed by filtration through Whatman 1 filter paper. Extracts were frozen until analysis. Extracts were analyzed for $\text{NO}_3\text{-N}$ using a microplate colorimetric technique based on the Greiss reaction (Doane and Horwath, 2003) and for $\text{NH}_4\text{-N}$ using a microplate colorimetric technique based on the Berthelot reaction (Sims et al., 1995). Analysis of variance (ANOVA) was performed to identify differences in N concentrations across treatments using a mixed model utilizing treatment as a fixed effect and a random block effect. Data were analyzed separately by year due to a significant treatment by year interaction. Data were log-transformed prior to analysis to meet assumptions of normality, and mean separation was performed using Tukey's adjustment. All analyses were performed in R statistical software (R Development Core Team, 2019).

Results

Supplemental Table S2. Soil inorganic nitrogen (N) concentration (mg kg^{-1} dry soil \pm 1 standard error) in perennial forage cropping treatments in the second (2018) and third (2019) growing seasons following establishment in southeast Pennsylvania, USA. Soil samples were collected annually in June.

<u>Treatment</u>	<u>Soil inorganic N (mg kg^{-1} dry soil)</u>	
	<u>2018</u>	<u>2019</u>
Tall fescue (TF)	10.4 \pm 0.9 ab†	5.3 \pm 0.7 ab
Orchardgrass (OG)	9.5 \pm 0.5 b	4.3 \pm 0.7 b
White clover (WC)	15.3 \pm 1.5 a	5.7 \pm 0.4 a
TF + OG	9.3 \pm 0.9 b	4.6 \pm 0.4 ab
TF + WC	13.0 \pm 1.7 ab	4.9 \pm 0.4 ab
OG + WC	12.7 \pm 1.6 ab	4.8 \pm 0.2 ab
TF + OG + WC	12.6 \pm 2.6 ab	4.4 \pm 0.5 ab

†Lowercase letters indicate differences among values within each column based on mean separation using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$.

References

- Doane, T.A., and W.R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. *Analytical Lett.* 36: 2713–2722.
- Sims, G.K., T.R. Ellsworth, and R.L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. *Commun. Soil Sci. Plant Anal.* 26(1&2): 303–316.