

Legacy effect of legume proportion in the preceding ley on the bacterial community composition under a *Lolium multiflorum* following crop

Fox A.^{1,2}, Lüscher A.¹ and Widmer F.²

¹Agroscope, Forage Production and Grassland Systems, 8046 Zürich, Switzerland; ²Agroscope, Molecular Ecology, Reckenholzstrasse 191, 8046 Zürich, Switzerland

Abstract

Sustained legume-induced legacy effects of sown grassland mixtures on the performance of a following crop have been previously demonstrated, though it is currently unclear if any such effects are observed in the soil microbiome. In March 2015, following the completion of the AnimalChange mixtures experiment (2012-2014), the first soil samples were taken. These swards were then completely removed and all plots were reseeded with a *Lolium multiflorum* monoculture. Soil samples were subsequently taken in June, August and September. The bacterial 16S rRNA gene was PCR amplified from all samples and an amplicon-based Illumina Miseq sequence analysis conducted. In March, when legumes were still present, a highly significant effect of legume proportion was observed on the composition of the bacterial community ($P < 0.01$). Interestingly, even after re-seeding with *Lolium multiflorum*, a significant legacy effect between the former legume and non-legume monocultures was seen in the bacterial community in the following June and August ($P < 0.05$), with the effect disappearing at the September sampling ($P > 0.05$). Such legume-induced legacy effects on the microbiome may represent an important functional component of the increased performance of following crops in rotations, which utilize a legume component in the preceding ley.

Keywords: legacy effect, legumes, following crop, bacterial community composition

Introduction

It is known that sustained legacy effects on the composition of the soil microbiome can be induced by agricultural management (e.g. manure application, Zhang *et al.*, 2018) and also by environmental perturbations (e.g. Banerjee *et al.*, 2016). What remains unclear, however, is how the influence imparted by a plant functional group on the structure and functionality of the soil microbiome can endure even after that group's complete removal from a system. A recent study described a positive legume-induced, soil transferred, legacy effect on the sward performance of a following crop of *Lolium multiflorum* (Fox *et al.*, 2018). The present study aimed to gain additional insights into the persistence of this legume-induced legacy effect by investigating its impact on the bacterial community composition from the same experiment.

Materials and methods

Under the auspices of the EU-project AnimalChange, a field mixtures experiment was established at Zürich-Reckenholz (47°26'12' N, 8°31'51' E), conducted on a cambisol (top soil 42% silt, 26% clay, pH = 7.1) over a period of three years (2012-2014). The experiment utilized four model plant species, one grass (*Lolium perenne* L., cultivar (cv.) Alligator) and one forb (*Cichorium intybus* L., cv. Puna II) and two legumes (*Trifolium repens* L., cv. Hebe and *Trifolium pratense* L., cv. Dafila). The experiment contained plots with a sown legume proportion of 0, 50 and 100% grown on plots 3×5 m in size (for more details see Hofer *et al.*, 2016). Following the completion of the AnimalChange experiment, soil samples were taken for microbial analysis on 16 March 2015. In the spring of 2015 (21 April), these preceding swards were completely removed using a glyphosate herbicide and all plots were re-seeded with

a *Lolium multiflorum* L. monoculture. This sward was allowed to become established and soil samples were subsequently taken three times during 2015 (10 June, 25 August and 29 September). Soil DNA was extracted from all collected samples and the bacterial 16S rRNA gene (V3-V4 region) was PCR amplified and an amplicon-based Illumina Miseq sequence analysis conducted (Frey *et al.*, 2016). Raw sequence reads ran through a customized bioinformatic pipeline and a bacterial operational taxonomic unit (OTU) table was constructed. The raw data were imported into the R statistical software package where they were subjected to a square root transformation, converted to relative abundance and a Bray-Curtis dissimilarity matrix was constructed. To take into account the differing replicate number between the sown legume proportions, the dissimilarity matrix was sub-sampled to the lowest number of replicates 1000 times, and a PERMANOVA analysis (9999 permutations) was conducted on each, with the mean *P* value taken. Group centroids were calculated via PERMDISP analysis.

Results and discussion

At the March sampling, when the legumes were still present, there was a highly significant effect of sown legume proportion on bacterial community composition ($P < 0.01$). The 100% legume proportion harboured a significantly distinct bacterial community compared to the non-legume control ($P < 0.02$, distance between centroids = 0.056), while the 50% legume proportion was not significantly distinct from either the 0 (distance between centroids = 0.038) or 100% (distance between centroids = 0.019) legume proportions (both $P > 0.05$). This would suggest a gradual shift in the composition of the microbiome as the composition of the plant community changes from non-legumes to legumes (Figure 1).

Interestingly, at the first sampling after the removal of the previous experimental swards in June, there was still a significantly distinct bacterial community associated with the former 100% legume proportion when compared with the former non-legume treatment ($P < 0.05$, distance between centroids = 0.038). This effect again persisted into the next sampling in August ($P < 0.05$, distance between centroids = 0.033). At the final sampling event in September, six months after the destruction of the previous AnimalChange sward, the bacterial community composition between the previous legume and non-legume monocultures was no longer significant ($P > 0.05$, distance between centroids = 0.027).

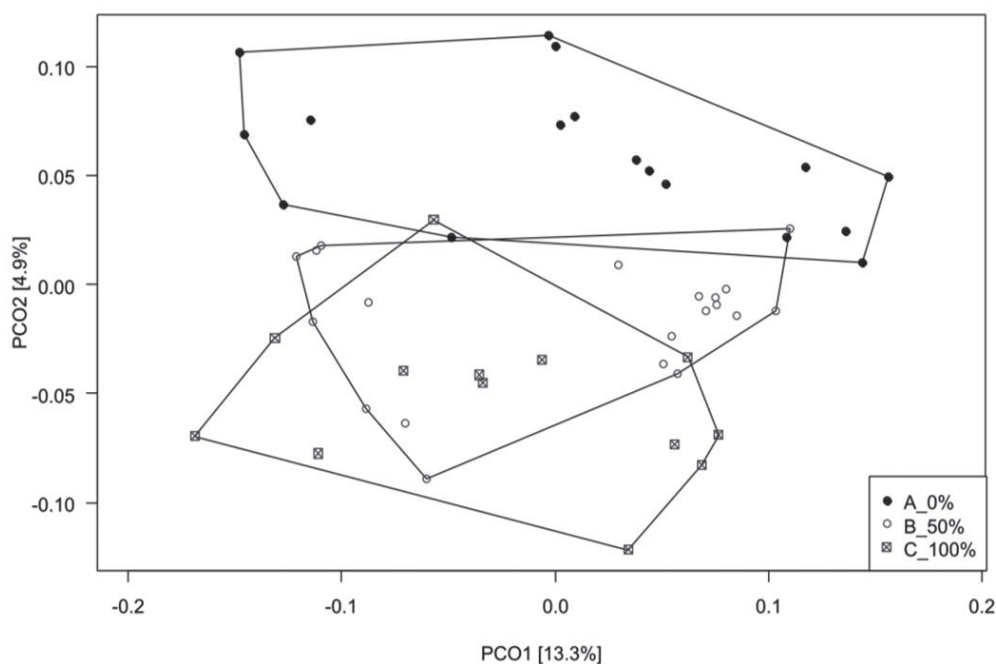


Figure 1. PCoA plot of the bacterial community composition among the different legume proportions in the seed mix; 0 (black dot), 50 (white dot) and 100% (crossed square), when the swards of the AnimalChange experiment were still present in March. Convex hulls indicate the different groupings of the bacterial community from sown legume proportions.

There are a few possible mechanisms behind this observation. Firstly, rhizobacteria may have remained attached to legume roots which persisted in the soil matrix. The composition of the bacterial community may also have adapted to utilize the changing quality of the soil organic matter (SOM and litter) due to legume presence, as these plants are known to have a high N content and a low C/N ratio (Nyfeler *et al.*, 2011). Hammelehle *et al.*, 2019 found that after a two years cultivation of a clover-grass mixture, the residual N potential of clover derived symbiotically fixed nitrogen in the soil was 10 g m⁻². Thus, the legacy effect observed may be a result of the soil bacteria continuing to utilize these legume-derived residues as a nutrient source. Intriguingly, these results would also suggest a role played by soil bacteria in the previously reported positive legume legacy effect on following crop performance (Fox *et al.*, 2018), though further research into the functionality of the bacterial community is necessary to establish any cause and effect mechanism.

Conclusion

These results demonstrate a legume-induced legacy effect on the composition of the soil bacterial community. This observation builds on (and may well be an explanatory mechanism for) the previous observation of the legume-induced legacy effects on following crop performance.

Acknowledgements

Funding was received from both the European Union's Seventh Framework Programme (FP7/2007 2013; grant agreement no. 266018) and the Swiss National Science Foundation (Biodiversa).

References

- Banerjee, S., Helgason, B., Wang, L., Winsley, T., Ferrari, B.C. and Siciliano, S.D. (2016) Legacy effects of soil moisture on microbial community structure and N₂O emissions *Soil Biology and Biochemistry* 95, 40-50.
- Fox, A., Suter, M., Widmer, F. and Lüscher, A. (2018) Yield stimulating legacy effect of sown legume abundance in a ley on the performance of a *Lolium multiflorum* following crop *Grassland Science in Europe* 23, 60-62.
- Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F. and Hartmann, M. (2016) Microbial diversity in European alpine permafrost and active layers *FEMS Microbiology Ecology*, 92.
- Hammelehle, A., Oberson, A., Lüscher, A., Mäder, P. and Mayer, J. (2019) New insights in below ground nitrogen of clover-grass mixtures and its residual nitrogen potential for subsequent crops. *Grassland Science in Europe* 24 (this volume).
- Hofer, D., Suter, M., Haughey, E., Finn, J. A., Hoekstra, N.J., Buchmann, N. and Lüscher, A. (2016) Yield of temperate forage grassland is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology* 53, 1023-1034.
- Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E. and Lüscher A. (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155-163.
- Zhang, Y., Hao, X., Alexander, T.W., Thomas, B.W., Shi, X., Lupwayi, N. (2018) Long term and legacy effects of manure application on soil microbial community composition. *Biology and Fertility of Soils* 54, 269-283