

Long-term mineral fertilizer application strongly influences soil microbial community structure but not diversity

Fox A.^{1,2}, Schultz S.², Brennan F.¹, Widmer F.³, Huguenin-Elie O.⁴, Schloter M.² and Lüscher A.⁴

¹*Environment, Soils and Land Use, Teagasc, Johnstown Castle, Co. Wexford, Ireland;* ²*Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Ingolstaedter Landstrasse 1, Munich, Germany;* ³*Molecular Ecology, Agroscope, Reckenholzstrasse 191, Zürich, Switzerland;* ⁴*Forage Production and Grassland Systems, Agroscope, Reckenholzstrasse 191, Zürich, Switzerland*

Abstract

Mineral fertilizer application is a common management practice in grassland production. However, its potential long-term influence on soil microbial communities requires further study. In 1972, a split-plot experiment was established in the Jura region of Switzerland containing a non-fertilized control and a fertilized treatment of 150, 80 and 240 kg ha⁻¹ yr⁻¹ mineral N, P and K, respectively. Soil samples for microbial community analysis were taken in the summer of 2018. There was a highly significant effect of mineral fertilizer application on both soil fungal and bacterial community structure (all $P < 0.001$), with unique fungal and bacterial indicator OTU (operational taxonomic unit) being associated to each treatment. Contrastingly, alpha diversity measures (OTU richness and inverse Simpson index) were not affected ($P > 0.05$). These results demonstrated that long-term mineral fertilizer application had a strong influence on soil microbial community structure, promoting different microbial taxa, but did not affect the overall alpha diversity of these communities.

Keywords: permanent grassland, mineral fertilizer, soil fungi, soil bacteria

Introduction

Grassland management intensity strongly influences soil microbial communities, as has recently been demonstrated by comparing intensively and extensively managed grasslands in multiple European countries (Fox *et al.*, 2021). Grassland management, however, encompasses numerous individual practices administered throughout the growing season, such as mineral and/or organic fertilizer application, utilizations (cuttings and grazing events), reseeding and herbicide application. Thus, further study is required to elucidate the influence that such individual practices have on soil microbial communities. This is a critical knowledge gap, as the soil microbiome is fundamental in a number of agroecosystem processes, such as soil nutrient cycling, organic matter decomposition and plant productivity (Bertola *et al.*, 2021). In this study, we take one individual aspect of grassland management, mineral fertilizer application, and examine its long-term influence on: (1) soil fungal and bacterial community structure; and (2) soil fungal and bacterial alpha diversity measures.

Materials and methods

In 1972, a long-term experiment was established in the Jura region of Switzerland (930 m, 242' 090/617' 120). Included was a non-fertilized control (Con) and a fertilized treatment which received 150, 80 and 240 kg ha⁻¹ yr⁻¹ of mineral N, P and K, respectively (NPK, both n=6, 2-cut and 3-cut variants pooled together). Soil samples were taken in July 2018 (46 years after commencement), with eight cores being taken per plot, using a soil auger (ø 2.5 cm) to a depth of 20 cm. Each sample was homogenized and soil DNA extracted. The fungal internal transcribed spacer region (ITS2) and the bacterial 16S rRNA gene were PCR amplified as community markers and an amplicon-based Illumina Miseq sequence analysis conducted (Frey *et al.* 2016; Tedersoo *et al.* 2014). Operational taxonomic units (OTU) were clustered at 97% identity, and OTU richness and the inverse Simpson alpha diversity measures were calculated using the 'summary.single' command in MOTHUR (version 1.36.1). Taxonomic assignment was done

using a database extracted from NCBI for fungal sequences and the SILVA database (version 132) for bacterial sequences. The effect of treatment on both fungal and bacterial community structure was tested with PERMANOVA using the 'adonis2' function (vegan package), and on the alpha diversity measures using analysis of variance. Associations of OTU to each treatment was determined by correlation-based indicator species analysis ('multiplatt' function), with an indicator OTU being defined as having an IndVal = 1.0 and a P -value ≤ 0.05 . Analysis was done using R statistical software, version 4.0.2.

Results and discussion

There was a highly significant effect of mineral fertilizer application on both soil fungal and bacterial community structure (both $P < 0.001$, Figure 1, panels A and B, respectively). The effect size was bigger for soil fungi than for soil bacteria, as was seen in the R^2 of the model (0.449 compared to 0.301, respectively) and the community centroid distance (0.570 compared to 0.255, respectively). Such a pronounced effect is in line with recently reported differences in soil fungal and bacterial community structures between extensively and intensively managed grasslands in European grasslands (Fox *et al.*, 2021).

Additionally, different indicator microbial taxa were associated to both Con and NPK, supporting the notion that contrasting nutrient inputs in grasslands select for specific microbial groups (Leff *et al.* 2015). A higher number of indicator fungal OTU were associated to Con (51) than to NPK (27), while the same number of indicator bacterial OTU were associated to both treatments (7). Among the indicator fungal OTU associated to Con, were those assigned to the genera *Clavaria* and *Leobhumicola*, which have previously been shown to be indicator fungi for extensively managed grasslands in Europe (Fox *et al.*, 2021, McHugh *et al.*, 2001).

In contrast to what was observed for community structure, there was no significant difference ($P > 0.05$) between Con and NPK in terms of fungal OTU richness (avg. 654 and 653 OTU, respectively), bacterial OTU richness (avg. 1,828 and 1,973 OTU, respectively), fungal inverse Simpson index or bacterial inverse Simpson index (Figure 2). The lack of a treatment effect on fungal alpha diversity is somewhat surprising, as fungal OTU richness has been reported as being lower in intensively managed grasslands, compared to extensively managed grasslands, in many European regions (Fox *et al.*, 2021). Therefore, this reported decline in fungal OTU richness may not solely be due to mineral fertilizer application, but rather other aspects of grassland management and/or their interactive effects. These results indicate that while mineral fertilization significantly affects the abundance of individual microbial taxa, it does not influence microbial community diversity.

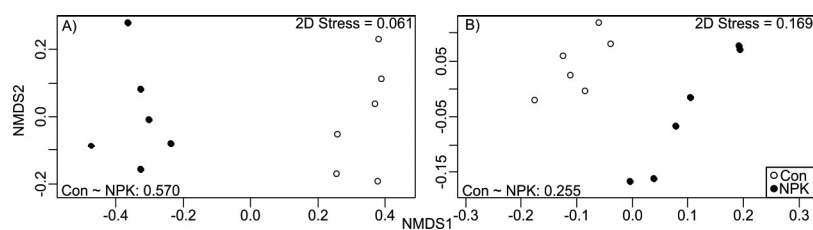


Figure 1. A non-metric multidimensional scaling plot (NMDS) displaying the difference in both fungal (panel A) and bacterial (panel B) community structure between the non-fertilized control (Con, open symbols) and the fertilized NPK treatment (closed symbols). Also displayed in each panel is the community centroid distance between treatments (Euclidian distance).

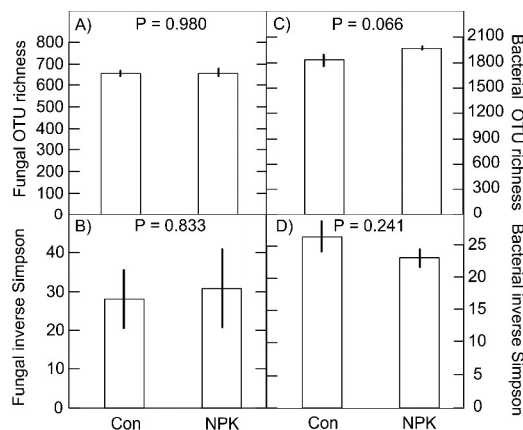


Figure 2. Effect of mineral fertilizer application (± 1 standard error) on (A) fungal OTU richness, (B) fungal inverse Simpson index, (C) bacterial OTU richness and (D) bacterial inverse Simpson index.

Conclusions

Our study demonstrates the strong, highly significant effect that mineral fertilizer application has on soil microbial community structure, suggesting that it is a major driver behind the recently reported differences in soil microbial community structure between grasslands of highly contrasting management types in Europe. The lack of effect mainly on fungal alpha diversity measures, however, would indicate that mineral fertilizer application alone may not explain the lower levels of fungal diversity previously reported in intensively managed European grasslands.

Acknowledgements

Funding was received through the Swiss National Science Foundation (grant no. 31BD30-172463), and the Research Leaders 2025 programme co-funded by Teagasc and the European Union's Horizon 2020 research and innovation programme (grant no. 754380).

References

- Bertola M., Ferrarini A. and Visioli G. (2021) Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by -omics approaches. *Microorganisms* 9, 1400.
- Fox A., Widmer F., Barreiro A., Jongen M., Musyoki M., Vieira Á. ... and Lüscher A. (2021) Small-scale agricultural grassland management can affect soil fungal community structure as much as continental scale geographic patterns. *FEMS Microbiology Ecology* 97, fiab148.
- 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(3), fiw018.
- Leff J.W., Jones S.E., Prober S.M., Barberán A., Borer E.T., Firn J.L. ... and Fierer N. (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the USA* 112, 10967-10972.
- McHugh R., Mitchel D., Wright M. and Anderson R. (2001) The fungi of Irish grasslands and their value for nature conservation. *Biology and Environment Proceedings of the Royal Irish Academy* 101B, 225-242.
- Tedersoo L., Bahram M., Põlme S., Kõljalg U., Yorou N.S., Wijesundera R., ... and Abarenkov K. (2014) Fungal biogeography. Global diversity and geography of soil fungi. *Science* 346(6213), 1256688.