TITLE

Linkages between aboveground and belowground community compositions in grasslands along a historical land-use intensity gradient

AUTHOR NAMES AND AFFILIATIONS

Safaa Wasofa\*, An De Schrijvera,b, Stephanie Schelfhouta, Michael P. Perringa,c, Elyn Remya, Jan Mertensa, Eduardo de la Peñad, Nancy De Suttere, Nicole Viaened,e, & Kris Verheyena

aGhent University - Department of Environment, Forest & Nature Lab (ForNaLab), Geraardsbergsesteenweg 267, B-9090 Melle-Gontrode, Belgium

bUniversity College Ghent, Faculty of Science and Technology, Brusselsesteenweg 161, B- 9090 Melle, Belgium

cEcosystem Restoration and Intervention Ecology (ERIE) Research Group, School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 AUSTRALIA

dDepartment of Biology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium.

eFlanders research institute for Agriculture, Fisheries and Food, Plant Sciences Unit, Burgemeester Van Gansberghelaan 96, B- 9820 Merelbeke, Belgium

\*Corresponding author: Safaa Wasof, E-mail: safaa.wasof@ugent.be

Abstract

*Background and aims* Improving our understanding of ecosystem responses to land-use intensification requires explicit consideration of linkages between aboveground and belowground communities. Here, we explore linkages between plant, soil microbial and nematode community compositions along a historical land-use intensity (hLUI) gradient.

*Methods* We used co-inertia analysis to investigate linkages between each paired community composition in 33 grasslands with similar hydrology and soil texture but contrasting hLUI and associated soil chemical properties (e.g. pH, phosphorus). We estimated the percentage cover of plant species, identified nematodes to genus level, and analysed the microbial community using phospholipid fatty acid (PLFA) profiling.

*Results* Plant and nematode communities were more strongly linked as compared to either community’s links with microbes, although all pairwise comparisons were significant. Linkage strength did not depend on the degree of hLUI. We found significant variations in plant and nematode, but not in microbial, community compositions along the hLUI gradient.

*Conclusions* Large changes in soil fertility associated with hLUI have led to shifts in vegetation community composition matched by changes in the composition of different soil communities, or *vice versa*. The nematode community seems to be more responsive to vegetation composition than other trophic groups. Additional research in an experimental setting will elucidate the mechanisms underpinning the observed relationships.

**KEYWORDS**

Co-inertia; microbes; nematodes; pH; phosphorus; species-rich grasslands

**TYPE OF ARTICLE**

Regular article

**ABBREVIATIONS**

COIA Co-inertia analysis

hLUI: historical land-use intensity

PLFA phospholipid fatty acid

AMF Arbuscular mycorrhizal fungi

HCPC Hierarchical clustering on principal components

PCA Principal component analysis

INTRODUCTION

Aboveground and belowground components of ecosystems are strongly interlinked at the community level (Wardle 2002; Bardgett and Wardle 2010). On the one hand, plant communities affect belowground biota communities both directly and indirectly (De Deyn and van der Putten 2005; Bardgett and Wardle 2010). Direct effects can be attributed to differences between plant communities in the quality and quantity of litter and root exudates supplying C to soil organisms (Wardle 2002). Indirectly, aboveground vegetation influences soil physiochemical properties such as pH, soil organic matter and soil structure (Bardgett and Wardle 2010; Kardol and De Long 2018). On the other hand, belowground fauna (e.g. soil microbes) can have profound effects on the composition and growth of plant communities by determining the supply of available soil nutrients (Wardle et al. 2004). Explicit consideration of these linkages between plant and belowground communities could improve our understanding of the consequences of global change, such as land-use change, on terrestrial ecosystems (Wardle 2002; Wardle et al. 2004). However, while numerous studies investigated relationships between these two components (e.g. Broughton and Gross 2000; Hooper et al. 2000; Korthals et al. 2001; Grayston et al. 2004; Jangid et al. 2011; Milcu et al. 2013), most studies have generally considered relationships between aboveground-belowground diversity, biomass and abundance (but see Roy-Bolduc et al. 2016; Cassman et al. 2016). Direct comparisons of plant community – belowground community compositional shifts along a gradient of increasing land-use intensity (LUI) remain scarce (Kardol et al. 2005; Cassman et al. 2016).

Land-use intensification, particularly the conversion of nutrient-poor semi-natural grasslands to nutrient-rich productive agroecosystems, was a common practice in North-West Europe (Kardol et al. 2005; Holtkamp et al. 2008). The application of large amounts of fertilizers and manure increased soil fertility and plant productivity and, consequently, affects competitive interactions between species (Socher et al. 2013). Increased LUI has led to biodiversity loss and homogenisation of communities at different trophic levels, both above- and belowground (Hooftman and Bullock 2012; Middleton 2013; Allan et al. 2014; Gossner et al. 2016). Besides driving a decline in species richness, land-use intensification is commonly viewed as a selective pressure causing shifts in aboveground and belowground community compositions by favouring species functionally adapted to nutrient input (Bardgett and Wardle 2010; Simons et al. 2017). For instance, high LUI promotes fast-growing, acquisitive plant species with high litter quality (low C:N ratio), over slow-growing species with a more conservative growth strategy (de Vries et al. 2006; Allan et al. 2015). Soil biota community composition is also affected by LUI. For instance, the ratio between bacteria and fungi changes towards a more bacteria-dominated system with increased intensification (e.g. added nutrients, more frequent and larger disturbance through ploughing) (Wardle 2002; de Vries et al. 2012). Therefore, changes in soil fertility and subsequent changes in the composition of the vegetation should be matched by corresponding changes in the composition of different element of the soil community (e.g. nematodes and microbes). Investigating the existence and strength of such compositional relationships may render critical information to the restoration of ecosystems, conservation of aboveground and belowground species and the services they provide to humanity (Hooper et al. 2000; Kardol et al. 2010; Marrs 2016).

In this study, we focussed on soil micro-organisms (bacteria and fungi), given their important role as key drivers of changes in plant communities (Reynolds et al. 2003; Van Der Heijden et al. 2008). We additionally studied nematodes, in separate functional groups, because they have important effects on plant communities and because they are abundant and taxonomically and functionally diverse, and are often used as an indicator of soil ecosystem structure and functioning (Bongers and Ferris 1999; Kardol et al. 2009; Kardol and De Long 2018).

Our first research question was “Are the community compositions of aboveground and belowground organisms interlinked in grasslands along a historical LUI (hLUI) gradient?”. Overall, we expected significant and strong linkages between the compositions of plants, soil microbiota and nematodes along the investigated gradient. Secondly, we asked “Do meso- and eutrophic grasslands have weaker above- and belowground compositional linkages than oligotrophic grasslands?” We expected that under soil nutrient limitation, the plant community is more dependent on the belowground community for nutrients, resulting in stronger ecological links between plant and belowground in oligotrophic grasslands compared to the ones in meso- and eutrophic grasslands. We investigated the significance and strength of linkages between each pair of communities by means of co-inertia analysis (COIA). COIA is a powerful, yet simple and promising, multivariate method of coupling large datasets (Dray et al. 2003), but has been very little employed in this context (but see for ex. Cassman et al. 2016). To further interpret our results, we assessed compositional changes along the gradient using non-metric multi-dimensional scaling (NMDS) and indicator species analyses.

**Materials & methods**

**Grassland selection and soil sampling**

We selected 33 permanent grasslands with a different historical land-use intensity (hLUI) (see Appendix S1 for a general description of the sites) due to past agricultural activity. Low hLUI means no or a low amounts of fertilizer were applied and included only one or two cuts of hay per year. High hLUI consisted of large amounts of fertilizers being applied annually (up to 70 kg phosphorus (P)/ha/year (Tits et al. 2016)), high mowing frequencies (up to six times per year) and high stocking rates (4 livestock units/ha (Vuylsteke et al. 2014)). The intensity of fertilization, and consequently the P-stocks in soils, strongly depended on a farmer’s productivity expectation, the farm size and its livestock density (Schröder et al. 2010). Active fertilization stopped in all grasslands between 5 and 33 years ago (Table S1 in Appendix S1), although they are all still exposed to atmospheric N-deposition of, on average, 28 kg N/ha/year (Cools et al. 2015). Current management consists of hay cutting and removal, often followed by late-seasonal grazing by cattle, horses or sheep. Based on the soil texture, region and hydrology, we can assume that, before intensification, these grasslands were *Nardus* grasslands (European Priority Habitat Type 6230). *Nardus* grasslands are dry or mesophile perennial grasslands on oligotrophic sandy or loamy soils, which are acidic to weakly acidic (pHH2O between 4.5 and 6) (De Graaf et al. 2009) and with bioavailable P concentrations between 1.5 and 14.1 mg POlsen. kg-1 soil (Schelfhout et al. 2017). These low productivity habitats are mainly dominated by mat-grass (*Nardus stricta*), cross-leaved heath (*Erica tetralix*) and tormentil (*Potentilla erecta*) (T’Jollyn et al. 2009). All sites have comparable hydrology and are located on the same parental soil material of glacial sandy deposits, in Flanders, Belgium (Table S1 in Appendix S1). Given these similarities, in our study, we assume that high hLUI resulted in eutrophic grasslands; medium hLUI resulted in mesotrophic grasslands; and, low hLUI resulted in oligotrophic grasslands.

In September 2013, soil samples were collected from the 33 grasslands. In most of the 33 grasslands, five 4 m² quadrats were laid out as subplots, except for the very small and homogenous sites (two or three subplots). For chemical analyses, in each subplot, five soil cores (0-15 cm) were collected from the four corners and the centre of the subplot. The soil samples were collected using a 3-cm-diameter soil auger, pooled together in a plastic bag, and transferred to the laboratory as soon as possible. In the laboratory, soil samples were dried (40 °C for 48 hours), sieved (2 mm mesh size), and chemically analysed (see *Soil chemical analyses*). Soils for PLFA and nematode analyses were sampled by taking five soil samples of 15 cm deep from all subplots following the same procedure as described above, but samples were stored at a maximum of 4°C before taking them to the lab for further processing (see *Response variables*). Here, fresh soil was also sieved over a 2mm sieve.

**Soil chemical analyses**

We measured chemical parameters that are related to soil acidification (pHKCl, exchangeable Al3+ and Ca2+) and to the fertilisation history of the sites (total and bioavailable P). pHKCl was measured using a glass electrode (Orion, model 920A) after extracting 14 mL of soil in a 70 mL KCl (1 *M*) solution. Soil exchangeable Al3+ and Ca2+ concentrations were determined by atomic absorption spectrometry (AA240FS, Fast Sequential AAS) after extracting 5 g of dry soil in 100 ml BaCl2 (0.1 *M*). Total soil P concentration (PTotal) was determined according to the colorimetric malachite green procedure (Lajtha et al. 1999) after acid wet digestion of 0.2 g soil with HClO4/HNO3/H2SO4 in Teflon pots at 150 °C for 4 h. As a measure of bioavailable P, 2 g dry soil was extracted in 0.5 M NaHCO3 for 30 min at pH 8.5 (POlsen) and subsequent colorimetric analysis of the extracts using the malachite green procedure. All measurements at the subplot level were then averaged to the site level prior to statistical analyses to avoid pseudoreplication. We did not consider soil N-concentrations because in non-organic sandy soils this element is known to be highly mobile as nitrate and to quickly disappear after cessation of fertilisation (Van Der Woude et al. 1994). In addition, other studies that were conducted in the same studied regions found that these grasslands were N-limited (Schelfhout et al. 2015, 2017).

**Response variables**

We collected soil samples for microbial and nematodes analyses at the same time as carrying out vegetation surveys.

*Vegetation survey*

In each subplot (4 m² quadrat), we identified plant species and estimated their relative cover as the percent (%) area of the quadrat occupied by a plant species. Then, we averaged these values to get the relative cover at the site level. In total, we identified 76 plant species (see AppendixS2 for the list of studied plant species).

*Microbial soil community*

Microbial community composition was determined by analysing the ester-linked phospholipid fatty acid (PLFA) composition of the soil using a chloroform-methanol-water extraction phase ratio based on the method of Bligh and Dyer (1959), as modified by White et al. (1979). Because different subsets of the soil community have different “signature” fatty acids (Tunlid and White 1991), determination of PLFA patterns has become one of the most commonly used methods to study microbial community structure (Frostegård et al. 2011). Specifically, individual PLFAs were used as biomarkers to measure the relative abundance of active fungi and bacteria (Bardgett et al. 1996), which constitute some 90–95% of total heterotrophic metabolism in most soils (Petersen and Luxton 1982). For each site, the abundance of individual fatty-acid methyl-esters was expressed as the proportion (mol %) of the sum of all fatty acids. Fatty acid nomenclature was used as described by Frostegård et al. (1993) and other references (see Appendix S3 for PLFA markers used for taxonomic microbial groups and for the used references). The fatty acids were summed up to estimate Gram-negative (GM-) bacterial, Gram-positive (GM+) bacterial, actinomycetes, fungal, and arbuscular mycorrhizal fungal (AMF) biomass (Appendix S3). These taxonomic microbial groups were then used in further analyses.

*Nematodes community counts and identification*

We measured the abundance and diversity of nematodes by extracting all nematodes from 100 cm3 of soil by zonal centrifugation (Seinhorst 1966; Hendrickx 1995; Wander et al. 2007). After centrifugation, nematodes were collected in about 125 mL of water with traces of MgSO4 and kaolin. The suspension was left in the 150-mL beaker for 3 h, allowing the nematodes to settle to the bottom. Then, the supernatant was gently removed and the total number of nematodes in the sample was counted using a dissecting microscope in a final volume of 30 mL. After counting, the nematode suspension was transferred into a conical tube. After settling down of the nematodes, 25 ml of the supernatant was removed with a glass pipet. The nematodes in the remaining 5 ml were fixed by adding 10ml of 4% warm (60-80°C) formaldehyde. Then, 1 mL of the resulting suspension was mounted on large microscope glass slides (50 × 76 mm). The first 100 nematodes encountered on different slides (5 slides on average) from each sample were identified to genus level following the identification keys of Bongers and Bongers (1998), and subsequently divided into trophic groups (Yeates et al. 1993): herbivores, bacterivores, fungivores, omnivores, and predators. The number of nematodes of each different genus for each sample was extrapolated from this subsample of 100 nematodes.

**Statistical analysis**

To investigate the linkages between vegetation, soil microbial and nematode community compositions along the hLUI gradient (i.e. our first question), we analysed each paired community datasets by means of co-inertia analysis (hereafter COIA) (Dolédec and Chessel 1994; Dray et al. 2003). COIA is a very general and flexible multivariate method that describes the relationships between two datasets that share the same samples (sites in our case), by searching successive pairs of axes with maximum covariance (instead of correlation, as in canonical correlation analysis) (Dolédec and Chessel 1994; Dray et al. 2003). This method finds a common space into which the sites and indicators of both datasets can be projected and compared (the distance between sites measures their similarity). Prior to COIA analysis, Principal Component Analysis (PCA) was performed on Hellinger-transformed ecological community datasets. The strength of the coupling between each paired table was evaluated with the RV coefficient, the “Correlation [R] of (multivariate) Vectors”, which is a multivariate extension of the Pearson correlation coefficient (Escoufier 1973). This coefficient gives a measure of global similarity of the two datasets and takes a value between 0 and 1: the closer the coefficient approaches to 1, the stronger the correlation between the two datasets. We then used Monte-Carlo tests (with 999 random permutations) to assess the significance of the correlations. Co-inertia analysis and Monte-Carlo tests were performed with the “ade4” library of the R package using the functions “coinertia” and “randtest”, respectively (Dray and Dufour 2007; R Core Team 2017). To assess how community composition relationships varied according to soil chemical properties, we overlaid the soil variables (pHKCl, exchangeable Al3+ and Ca2+, total P and POlsen) on the COIA plots as vectors, using the ‘envfit’ function in the ‘vegan’ package (Oksanen 2015). The direction and length of each vector indicate the direction of the gradient and the strength of the correlation, respectively. The significance of the fitted vectors was assessed by a permutation procedure (999 permutations). The goodness-of-fit statistic is the squared correlation coefficient (r²).

In order to answer our second question, we first clustered the 33 sites on soil variables described above using Hierarchical Clustering on Principal Components (HCPC) (R Core Team 2017). HCPC combines PCA and a posterior clustering process based on a mixed algorithm (Ward’s classification method with the K-means algorithm) (Husson et al. 2010; Argüelles et al. 2014). HCPC was performed on data with the FactoMiner R package and default parameters using the function “HCPC”. The resulting soil group clusters (n = 3) from this analysis (**Fig. 1a**) were assigned the relative terms ‘Eutrophic’, ‘Mesotrophic’ and ‘Oligotrophic’. The eutrophic soil group aligned with high concentrations of total P, POlsen, and high pHbut with low concentrations of exchangeable Al; whereas the Oligotrophic soil group showed the opposite trends (**Fig. 1b-c**). Mesotrophic soils adopted an overall intermediate position in soil chemical properties, which was visualised in their position around the origin of the PCA (**Fig. 1b-c**).

We then used these soil groups to answer our second question, that is to assess if the linkages between vegetation, soil microbial and nematode community compositions were weaker in the Mesotrophic and Eutrophic soil groups compared to their linkages in the Oligotrophic grasslands. For this purpose, we used the co-ordinates of the COIA projections of sites onto the first two COIA axes and calculated the Euclidean distance between them. For instance, the co-ordinates of the COIA projection of sites from each pair of datasets (say vegetation and microbial datasets) are graphed in a bi-plot and are connected by an arrow. The beginning of the arrow is the position of the site described by one set of indicators (e.g. vegetation); the end of the arrow is the position of the site described by another set of indicators (e.g. the microbes). The strength of correlation between two datasets, for each site, is inversely correlated with the length of the arrow: the shorter the arrow, the better the concordance between the two datasets. For this reason, we calculated the length of the arrows of sites in each soil group (i.e. Oligo-, Meso- and Eutrophic soil groups). We then used ANOVA analysis and Tukey’s HSD tests to compare among group means, making sure homoscedasticity assumptions were met.

In order to investigate whether the three soil group clusters display variations in plant, soil microbial and nematode community compositions, we used nonmetric multidimensional scaling (NMDS) implemented in the ‘metaMDS’ function from the Vegan R package. We assessed differences in community composition among soil groups for the different taxa using Permutational Analysis of Variance (PERMANOVA) in the ‘adonis’ function. We used Bray-Curtis distance matrices, based on Hellinger-transformed datasets for both procedures. Furthermore, in order to identify plant and soil biota taxa associated with a particular soil group cluster, we used the corrected Pearson’s phi coefficient of association (“r.g”; 9,999 permutations) as implemented by the ‘multipatt’ function from the R package indicspecies. The analysis produces the set of indicator species significantly associated to individual soil group or soil group combination.

**Results**

**Linking plant, soil microbial and nematode community compositions**

Overall, we found significant associations between plant and microbiota (*p*-value = 0.04; **Fig. 2**), plant and nematode (*p*-value = 0.001; **Fig. 3**), and microbiota and nematode (*p*-value = 0.04; **Fig. 4**) communities. These associations were strong between plant and nematode (RV = 0.41), but rather weak between plant and microbiota (RV = 0.18) and between microbiota and nematode communities (RV = 0.17). The first two co-inertia axes captured 96, 93, and 98% of the total variance in the plant-microbial, plant-nematode and microbial-nematode comparisons, respectively (**Figs. 2-4**), and thus presented a good initial summary of the concordance between the two datasets. Irrespective of the paired community datasets being analysed, all measured soil variables (pHKCl, exchangeable Al3+ and Ca2+, total P and POlsen) were significantly related to the first two axes of COIA, with pH being the most important variable (**Table 1**).

Arrows in panels (b) and (c) in **Figs. 2-4** represent the importance and direction of the contribution of indicators (e.g. plant species and microbial functional group in **Fig. 2b-c**) to the distribution of sites in the co-inertia space (panel (a) in **Figs. 2-4**). In our example, plant species and microbial functional groups projecting in the same direction from the origin have a strong association. There is a similar interpretation for plant and nematode indicators and for microbial and nematode indicators. We found consistent results among the different COIA analyses. Taken together, Eutrophic grasslands (sites depicted with red arrows in panel (a) of **Figs. 2-4)** were associated with plant species such as *Holcus lanatus, Lolium perenne* and *Ranunculus acris*, which were mainly associated with arbuscular mycorrhiza fungi (AMF) and with gram-positive (GM+) PLFA bacteria (**Fig. 2b-c**). Furthermore, plant-feeding nematodes (i.e. herbivorous) were associated with both *H. lanatus*, *L. perenne* and *R.* *acris* species (**Fig. 3**) and with AMF (**Fig. 4**). On the other hand, Oligotrophic grasslands (sites depicted with blue arrows in panel (a) of **Figs. 2-4**) were associated with plant species like *Molinia caerulea*, *Potentilla erecta*, *Caluna vulgaris* and *Agrostis canina*,which were mainly associated with gram-negative (GM-) PLFA bacteria and with fungal PLFA (**Fig. 2**). Moreover, bacterial-feeding and fungal-feeding nematodes were associated with the abovementioned plant species (**Fig. 3**)and with GM- bacteria and fungal PLFA (**Fig. 4**).

**Linkages between community compositions in the Mesotrophic and Eutrophic compared to the ones in the Oligotrophic grasslands**

The length of the arrows in panel (a) of **Figs. 2-4** is inversely correlated with the correlations between each paired compared dataset. Thus, the shorter the arrows, the better the concordance between the two datasets. Our results showed that irrespective of the studied pair of community compositions, there was no significant difference in mean arrow lengths between the Oligotrophic, Mesotrophic or Eutrophic grasslands (**Fig. 5**). Opposite to our expectation, this result suggests that linkages between community compositions are not stronger in the Oligotrophic compared to the ones in the Mesotrophic or in the Eutrophic grasslands.

**Community composition variations among the soil group clusters**

NMDS ordination and PERMANOVA revealed significantly different community composition in plants and soil nematodes among the soil groups clusters (i.e. Oligotrophic, Mesotrophic and Eutrophic) (PERMANOVA, P < 0.001), but no significant difference was found in soil microbial community (PERMANOVA, P = 0.2, R² = 0.11; Table S4-1 in Appendix S4). Both NMDS and indicator species analysis showed similar results as COIA. On the one hand, Oligotrophic grasslands were indicated by plant species characteristic of low hLUI grasslands (e.g. *Molinia caerulea*, *Potentilla erecta* and *Calluna vulgaris*), fungal PLFA (see Appendix S3 for PLFA markers used for fungal group) and omnivorous nematodes (Table S4-2 in Appendix S4). On other hand, Mesotrophic and Eutrophic grasslands were indicated by plant species characteristic of high hLUI grasslands (e.g. *Holcus lanatus*, *Taraxacum officinalis* and *Ranunculus acris*), arbuscular mycorrhizal fungi (AMF) and herbivorous nematodes (Table S4-2 in Appendix S4).

**Discussion**

In this study, we explored linkages between plant, soil microbial and nematode communities in grasslands over a historical land-use intensity (hLUI) gradient. While past research has also explored the influence of land-use intensification on aboveground and/or belowground community compositions (e.g. Kardol et al. 2005; Lauber et al. 2008; Van der Wal et al. 2009; Oehl et al. 2010), our study explicitly investigated the synchronous compositional responses of aboveground and belowground communities and quantified the strength and direction of such relationships. We expected that because of land-use intensification, large changes in soil fertility and concomitant shifts in the composition of the vegetation should be matched by corresponding changes in the soil community compositions. The co-inertia analyses demonstrated significant concordance between plant, soil microbial and nematode community compositional shifts along the investigated gradient. Plant and nematode communities were more tightly linked than either community with the microbial community. These linkages were not stronger in the Oligotrophic grasslands than in Mesotrophic (medium-level intensity) or Eutrophic (high-level intensity) grasslands. We found significant variations in plant and nematode, but not in microbial, community compositions between the three grasslands types representing different degree of hLUI.

Co-inertia analysis revealed significant relationships between community compositions along the hLUI gradient, which could occur for several reasons. First, significant linkages could be the result of similar compositional responses of aboveground and belowground communities to the same soil parameters. For instance, land-use change and intensification are known to have a significant effect on soil properties such as pH, and nutrient contents, in particular the availability of phosphorus (Oehl et al. 2010; Rousk et al. 2010; Ramirez et al. 2010). This in turn, significantly influences the composition of both aboveground and belowground communities (Moora et al. 2014; Rumpel et al. 2015). Our results showed that pH was the most important parameter explaining plant, microbial and nematode community datasets. This may be due to the potential toxicity of Al in acid soils (see further). Second, significant links could suggest that indirect effects of land-use intensification on belowground community compositions are observed due to changes that occur in plant community composition, or *vice versa*, as we found that both plant and nematode community compositions significantly differ between the different degrees of hLUI. Importantly, the co-inertia analysis is not a proof of causality and it is beyond the aim of this study to know which community is more influenced by the altered soil conditions and hence potentially affecting another community. Based on increasing evidence raised by previous studies on the role aboveground and belowground communities could exercise on each other (Bardgett et al. 1999; De Deyn et al. 2004; Van Der Heijden et al. 2008), we here assume that both communities could be powerful mutual drivers, with both positive and negative feedbacks (Wardle et al. 2004).

We found only weak coupling between the composition of the microbial community and both plant and nematode communities. Contrary to plant and nematode communities, we found no significant variation in microbial community composition between the three grasslands types. Absence of significant changes in microbial community composition between the different grasslands types might explain the lack of strong correlations between microbiota and both plants and nematodes in this study. However, when the different types of grasslands were compared with respect to their mean relative abundance of various microbial functional groups of PLFA, the impact of hLUI was clearer – Oligotrophic grasslands have significantly higher fungal PLFA, fungal/bacterial ratio (F:B) and lower AMF than Mesotrophic and Eutrophic grasslands. On the other hand, bacterial PLFAs (i.e. Actinomycetes. G− bacteria and G+ bacteria) did not significantly differ between the different grassland types (results not shown). These results suggest that changes in microbial communities could not be entirely explained by changes in vegetation and that other factors are at play (especially abiotic soil properties). In our study, we gave special attention to soil chemical characteristics (such as, pH and P) (factors shown to be important in structuring microbial communities; Fierer 2017), however, other uncharacterized environmental factors (such as soil moisture availability and temperature) could also be important in driving the microbial community composition (Nunan et al. 2005; Fierer 2017). Importantly, in this study, we identified communities to different levels. Plants were identified at the species level, nematodes at the genus level and microbiota were grouped in functional groups. Shifts in individual species’ abundances within the same functional group are possible, and that could explain why we were not able to detect strong correlations between the studied communities at the levels used. Lack of strong correlations between the two communities could also occur if some groups show low degree of specialization to specific soil conditions and land-use intensity level. For instance, in our study, gram-negative (GM-) PLFA bacteria were, in absolute numbers, the most abundant functional group regardless of the soil groups (i.e. Oligo-, Meso- and Eutrophic soils) (results not shown). High abundance of GM- bacteria in all grasslands and insignificant variation in bacterial PLFAs along the investigated gradient might suggest that bacterial organisms are less sensitive to environmental changes and may rapidly adapt to prevalent conditions than other microbial groups (e.g. fungi). Interestingly, linkages between plant and nematode community compositions were stronger than the linkages between either community with microbial community. Our results indicate that the nematode community may respond relatively faster to altered abiotic conditions and changes in plant community composition than other trophic groups in the soil foodwebs (Korthals et al. 2001; Kardol et al. 2005).

We found consistent results among all performed analyses (i.e. COIA, NMDS, indicator species analysis). Taken together, our results showed significant shifts in the community compositions from one composed of slower-growing plant species, fungi and bacterial- and fungal-feeding nematodes to one consisting of fast-growing plant species, AMF and plant-feeding nematodes as historical land-use intensification increased. Furthermore, the co-inertia analyses allowed us to identify groups of organisms that have strong associations. Among others, our study showed positive association (species pointing in the same directions in Fig. 2b-c) between plant species (such as *Trifolium repens* (white clover) and *Lolium perenne* (ryegrass)) and non-specific and GM+ bacterial PLFAs. This result is consistent with other study reporting that these PLFAs (non-specific and GM+ bacteria) were the most abundant microbial PLFA groups in soil under ryegrass and white clover (Kušlienė et al. 2014). White clover exudes inorganic (NH4+) and organic N compounds with a low C:N ratio, which would stimulate bacteria over fungi (de Neergaard et al. 2002). Interestingly, we found negative association (species pointing in the opposite directions in Fig. 2b-c) between *Peduclaris sylvatica* (hemiparasite plant)and AMF. This result confirms the finding of other study showing direct and indirect negative effect of AMF on the performance of parasitic plants during co-infection of host plants (Li et al. 2013). More generally, we found that fast-growing plant species, such as *L*. *perenne*, *H. lanatus* and *T*. *officinalis*, were associated with GM+ bacteria and AMF and plant-feeding nematodes, whereas slow-growing plant species, such as *M. caerulea* and *P*. *erecta*, were associated with fungi and fungi-feeding and bacteria-feeding nematodes. These results confirm the general belief that during the transition from an acid nutrient-poor semi-natural grassland to a less acid, nutrient-rich system, a shift is expected from plant communities dominated by slow-growing conservative species to communities dominated by fast-growing acquisitive species (Bardgett and McAlister 1999; Bardgett and Wardle 2010). These compositional shifts along the investigated gradient and specific associations between plants and soil organisms could have important consequences for carbon (C) and nitrogen (N) flow and dynamics, and consequently nutrient cycling and C loss in soils (Metcalfe et al. 2011; De Vries et al. 2015). For instance, grassland restoration management that promote soil mycorrhiza fungi and the establishment of slow-growing plant species have been shown to enhance the rate of soil C and N accumulation (De Deyn et al. 2011). On other hand, fast-growing plants generally produce more litter, richer in nitrogen (N) but poorer in C rich structural compounds, which accelerates nitrogen mineralization and increases rates of organic matter decomposition (Scheurwater et al. 1998; Baptist et al. 2009; Metcalfe et al. 2011). Soil organisms (such as herbivorous bacteria) could also have an important role in C and N dynamics by selectively consuming fast growing-associated high quality root material and thus the excretion of plant material high in labile C and N (Bardgett and Wardle 2010; Metcalfe et al. 2011). Therefore, differences in C and N allocation and assimilations between the two communities with contrasting growth strategies (fast-growing *vs*. slow-growing) may have important consequences for carbon and nitrogen dynamics, including loss pathways (Kardol et al. 2006).

Interestingly, our results showed that AMF were indicators of Mesotrophic and Eutrophic soils and were associated with fast-growing plant species. This increased affinity of AMF to our Eutrophic grasslands may be explained by the fact that these soils contained only lower concentrations of exchangeable Al3+, in contrast to acidic Oligotrophic soils (pHH2O < 4) where high Al-concentrations can be toxic to both plants and microbes, such as AMF (Göransson et al. 2008; Seguel et al. 2013). For instance, in our study, while Eutrophic grasslands had concentrations of up to 103 mg Al3+ kg-1 soil (with a mean of 32), Oligotrophic grasslands ranged from 127 to 528 mg Al3+.kg-1 soil, with a mean of 303 mg Al3+ kg-1 soil. These values in Oligotrophic grasslands are even higher than the ones that already showed a negative effect on AMF colonisation in another study (e.g. Göransson et al. 2008). The abundance of AMF were significantly negatively related to Al-concentrations and positively related to pH in our study and were significantly lower in Oligotrophic grasslands compared to its abundance in Mesotrophic and Eutrophic grasslands (results not shown).

We expected linkages between plant, soil microbial and nematode communities to be weaker in Mesotrophic and Eutrophic soils compared to their links in poor-nutrient Oligotrophic grasslands. That is because under low levels of nutrient availability, the plant community is expected to be more dependent on belowground communities for nutrients (Van Der Heijden et al. 2008; Cassman et al. 2016). Contrary to our expectation, our results do not confirm this hypothesis. As explained above, this could be because some groups, like GM- bacteria are dominant irrespective of the identified soil groups. Another possible explanation is that plants growing in low-nutrient environments may employ morphological and/or chemical adaptation strategies other than symbiotic association with mycorrhiza for efficient nutrient acquisition (Dakora and Phillips 2002). This may include increased root-hair formation, root-cluster initiation and development and release of root exudates (Dakora and Phillips 2002; Lambers et al. 2006). Our results open the avenue for an experimental study aiming at testing the relative importance of these different strategies (indirect via microbial symbiosis *vs.* direct control of the rhizosphere) for the acquisition of nutrients required for plant growth under different environments (e.g. poor-nutrient habitats).

In summary, the co-inertia analysis provided evidence of significant linkages between plant, soil microbial and nematode community compositions along a hLUI gradient. These significant coordinated changes could suggest that indirect effects of hLUI on belowground community composition are observed due to changes that occur in aboveground community composition, or *vice versa*. Our results suggest that the nematode community is more responsive to these changes in vegetation composition than other trophic groups in the soil foodwebs (microbes in our case), or *vice versa*. Given the observational nature of our study, it is not possible to ascertain which community is being influenced by land-use intensification and concomitant changes in soil conditions, and hence influencing another community. We suggest that simultaneous processes are at play, which could lead to the observed coupled dynamics among plant, microbial and nematode communities, which in turn could also influence the soil properties. In this context, experimental studies that aim at exploring the direct and indirect effects of multiple assumed driving factors, and their interactions, in modulating a given community composition should be prioritised. This could be particularly interesting in the context of ecological restoration. For instance, restoration of species-rich ecosystems is constrained by multiple factors, including high residual nutrient concentrations of past fertilization, limited dispersal abilities of target plant community, and degraded and altered soil biota communities (Pywell et al. 2002, 2007). Studies in this context have generally focused on the impact of bioavailable soil N and/or P or on the role of belowground soil biota on plant community development. However, an integrative understanding of how nutrient availability and soil biota interact in modulating plant community diversity and composition should be investigated. This could be achieved by having, for example, different microbial communities inoculated in soils across a range of nutrient availabilities.

Acknowledgement

SW works as postdoctoral researcher on a project funded by the Flemish Fund for Scientific Research (FWO, n° G050215N). The authors thank the three anonymous reviewers for their constructive comments. We would like to thank Stéphane Dray, Noriko A. Cassman and Cajo Ter Braak for their adviceconcerning the use of the co-inertia analysis. We furthermore thank nature conservators Kris Van der Steen, Marc Smets, Christine Verscheure and Eckhart Kuijken for the permission to do research in their nature reserves, their help in selecting the study parcels and providing all necessary information on the history and management of the plots. We furthermore thank Lander Baeten for his comments on the first version of the study, Annelies Haegeman for her assistance in interpreting PLFA analyses and Luc Willems and Greet De bruyn for the chemical analyses of the soil samples.

**References**

Allan E, Bossdorf O, Dormann CF, et al (2014) Interannual variation in land-use intensity enhances grassland multidiversity. PNAS 111:308–13. doi: 10.1073/pnas.1312213111

Allan E, Manning P, Alt F, et al (2015) Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. Ecol Lett 18:834–43. doi: 10.1111/ele.12469

Argüelles M, Benavides C, Fernández I (2014) A new approach to the identification of regional clusters: hierarchical clustering on principal components. Appl Econ 46:2511–2519. doi: 10.1080/00036846.2014.904491

Baptist F, Tcherkez G, Aubert S, et al (2009) 13C and 15N allocations of two alpine species from early and late snowmelt locations reflect their different growth strategies. J Exp Bot 60:2725–35. doi: 10.1093/jxb/erp128

Bardgett RD, Hobbs PJ, Frostegård Å (1996) Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biol Fertil Soils 22:261–264. doi: 10.1007/BF00382522

Bardgett RD, Mawdsley JL, Edwards S, et al (1999) Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Funct Ecol 13:650–660. doi: 10.1046/j.1365-2435.1999.00362.x

Bardgett RD, McAlister E (1999) The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biol Fertil Soils 29:282–290. doi: 10.1007/s003740050554

Bardgett RD, Wardle DA (2010) Aboveground-belowground linkages : biotic interactions, ecosystem processes, and global change. Oxford University Press

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bongers T, Bongers M (1998) Functional diversity of nematodes. Appl Soil Ecol 10:239–251. doi: 10.1016/S0929-1393(98)00123-1

Bongers T, Ferris H (1999) Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol Evol 14:224–228. doi: 10.1016/S0169-5347(98)01583-3

Broughton LC, Gross KL (2000) Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia 125:420–427. doi: 10.1007/s004420000456

Cassman NA, Leite MFA, Pan Y, et al (2016) Plant and soil fungal but not soil bacterial communities are linked in long-term fertilized grassland. Sci Rep 6:23680. doi: 10.1038/srep23680

Cools N, Wils C, Hens M, et al (2015) Atmosferische stikstofdepositie en Natura 2000 instandhoudingsdoelstellingen in Vlaanderen . Verkennende gewestelijke ruimtelijke analyse van de ecologische impact , van sectorbijdragen en van de bijdrage

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47. doi: 10.1023/A:1020809400075

De Deyn GB, Quirk H, Oakley S, et al (2011) Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. Biogeosciences 8:1131–1139. doi: 10.5194/bg-8-1131-2011

De Deyn GB, Raaijmakers CE, van Ruijven J, et al (2004) Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106:576–586. doi: 10.1111/j.0030-1299.2004.13265.x

De Deyn GB, Van der Putten WH (2005) Linking aboveground and belowground diversity. Trends Ecol Evol 20:625–633. doi: 10.1016/j.tree.2005.08.009

De Graaf MCC, Bobbink R, Smits N a C, et al (2009) Biodiversity, vegetation gradients and key biogeochemical processes in the heathland landscape. Biol Conserv 142:2191–2201. doi: 10.1016/j.biocon.2009.04.020

de Neergaard A, Hauggaard-Nielsen H, Stoumann Jensen L, Magid J (2002) Decomposition of white clover (*Trifolium repens*) and ryegrass (*Lolium perenne*) components: C and N dynamics simulated with the DAISY soil organic matter submodel. Eur J Agron 16:43–55. doi: 10.1016/S1161-0301(01)00118-6

De Vries FT, Bracht Jørgensen H, Hedlund K, Bardgett RD (2015) Disentangling plant and soil microbial controls on carbon and nitrogen loss in grassland mesocosms. J Ecol 103:629–640. doi: 10.1111/1365-2745.12383

de Vries FT, Hoffland E, van Eekeren N, et al (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biol Biochem 38:2092–2103. doi: 10.1016/j.soilbio.2006.01.008

de Vries FT, Liiri ME, Bjørnlund L, et al (2012) Land use alters the resistance and resilience of soil food webs to drought. Nat Clim Chang 2:276–280. doi: 10.1038/nclimate1368

Dolédec S, Chessel D (1994) Co-inertia analysis: an alternative method for studying species-environment relationships. Freshw Biol 31:277–294. doi: 10.1111/j.1365-2427.1994.tb01741.x

Dray S, Chessel D, Thioulouse J (2003) Co-inertia analysis and the linking of ecological data tables. Ecology 84:3078–3089. doi: 10.1890/03-0178

Dray S, Dufour A-B (2007) The ade4 Package: Implementing the Duality Diagram for Ecologists. JSS J Stat Softw 22:

Escoufier Y (1973) Le Traitement des Variables Vectorielles. Biometrics 29:751–760. doi: 10.2307/2529140

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15:579–590. doi: 10.1038/nrmicro.2017.87

Frostegård Å, Bååth E, Tunlio A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol Biochem 25:723–730. doi: 10.1016/0038-0717(93)90113-P

Frostegård Å, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43:1621–1625. doi: 10.1016/j.soilbio.2010.11.021

Göransson P, Olsson PA, Postma J, Falkengren-Grerup U (2008) Colonisation by arbuscular mycorrhizal and fine endophytic fungi in four woodland grasses – variation in relation to pH and aluminium. Soil Biol Biochem 40:2260–2265. doi: 10.1016/J.SOILBIO.2008.05.002

Gossner MM, Lewinsohn TM, Kahl T, et al (2016) Land-use intensification causes multitrophic homogenization of grassland communities. Nature 540:266–269. doi: 10.1038/nature20575

Grayston S., Campbell C., Bardgett R., et al (2004) Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Appl Soil Ecol 25:63–84. doi: 10.1016/S0929-1393(03)00098-2

Hendrickx G (1995) An automatic apparatus for extracting free-living nematode stages from soil. Nematologica 41:308

Holtkamp R, Kardol P, van der Wal A, et al (2008) Soil food web structure during ecosystem development after land abandonment. Appl Soil Ecol 39:23–34. doi: 10.1016/j.apsoil.2007.11.002

Hooftman DAP, Bullock JM (2012) Mapping to inform conservation: A case study of changes in semi-natural habitats and their connectivity over 70years. Biol Conserv 145:30–38. doi: 10.1016/j.biocon.2011.09.015

Hooper DU, Bignell DE, Brown VK, et al (2000) Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. Bioscience 50:1049–1061. doi: 10.1641/0006-3568(2000)050[1049:ibaabb]2.0.co;2

Husson F, Julie J, Pages J (2010) Principal component methods-hierarchical clustering-partitional clustering: why would we need to choose for visualizing data? Tech Rep

Jangid K, Williams MA, Franzluebbers AJ, et al (2011) Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biol Biochem 43:2184–2193. doi: 10.1016/J.SOILBIO.2011.06.022

Kardol P, Bezemer TM, van der Putten WH (2006) Temporal variation in plant-soil feedback controls succession. Ecol Lett 9:1080–8. doi: 10.1111/j.1461-0248.2006.00953.x

Kardol P, Bezemer TM, Van Der Putten WH (2009) Soil organism and plant introductions in restoration of species-rich grassland communities. Restor Ecol 17:258–269. doi: 10.1111/j.1526-100X.2007.00351.x

Kardol P, Bezemer TM, van der Wal A, van der Putten WH (2005) Successional trajectories of soil nematode and plant communities in a chronosequence of ex-arable lands. Biol Conserv 126:317–327. doi: 10.1016/J.BIOCON.2005.06.005

Kardol P, De Long JR (2018) How anthropogenic shifts in plant community composition alter soil food webs. F1000Research 7:4. doi: 10.12688/f1000research.13008.1

Kardol P, Wardle DA, Bardgett RD, et al (2010) How understanding aboveground-belowground linkages can assist restoration ecology. Trends Ecol Evol 25:670–9. doi: 10.1016/j.tree.2010.09.001

Korthals GW, Smilauer P, Van Dijk C, Van Der Putten WH (2001) Linking above- and below-ground biodiversity: abundance and trophic complexity in soil as a response to experimental plant communities on abandoned arable land. Funct Ecol 15:506–514. doi: 10.1046/j.0269-8463.2001.00551.x

Kušlienė G, Rasmussen J, Kuzyakov Y, Eriksen J (2014) Medium-term response of microbial community to rhizodeposits of white clover and ryegrass and tracing of active processes induced by 13C and 15N labelled exudates. Soil Biol Biochem 76:22–33. doi: 10.1016/J.SOILBIO.2014.05.003

Lajtha K, Driscoll C, Jarrell W, Elliott E (1999) Soil phosphorus: characterization and total element analysis. In: Robertson G, Coleman D, Bledsoe C, Sollins P (eds) Standard soil methods for long-term ecological research. Oxford University Press, New York, pp 115–142

Lambers H, Shane MW, Cramer MD, et al (2006) Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. Ann Bot 98:693–713. doi: 10.1093/aob/mcl114

Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40:2407–2415. doi: 10.1016/j.soilbio.2008.05.021

Li A-R, Guan K-Y, Stonor R, et al (2013) Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root hemiparasitic Pedicularis species: do the fungal partners matter at low colonization levels? Ann Bot 112:1089–98. doi: 10.1093/aob/mct177

Marrs RH (2016) Ecological restoration: Soil microbes call the shots. Nat Plants 2:16117. doi: 10.1038/nplants.2016.117

Metcalfe DB, Fisher RA, Wardle DA (2011) Plant communities as drivers of soil respiration: pathways, mechanisms, and significance for global change. Biogeosciences 8:2047–2061. doi: 10.5194/bg-8-2047-2011

Middleton BA (2013) Rediscovering traditional vegetation management in preserves: Trading experiences between cultures and continents. Biol Conserv 158:271–279. doi: 10.1016/j.biocon.2012.10.003

Milcu A, Allan E, Roscher C, et al (2013) Functionally and phylogenetically diverse plant communities key to soil biota. Ecology 94:1878–1885

Moora M, Davison J, Öpik M, et al (2014) Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiol Ecol 90:609–621. doi: 10.1111/1574-6941.12420

Nunan N, Daniell TJ, Singh BK, et al (2005) Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. Appl Environ Microbiol 71:6784–92. doi: 10.1128/AEM.71.11.6784-6792.2005

Oehl F, Laczko E, Bogenrieder A, et al (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol Biochem 42:724–738. doi: 10.1016/J.SOILBIO.2010.01.006

Oksanen J (2015) Multivariate analysis of ecological communities in R: vegan tutorial

Petersen H, Luxton M (1982) A Comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39:288. doi: 10.2307/3544689

Pywell RF, Bullock JM, Hopkins A, et al (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. J Appl Ecol 39:294–309. doi: 10.1046/j.1365-2664.2002.00718.x

Pywell RF, Bullock JM, Tallowin JB, et al (2007) Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. J. Appl. Ecol. 44:81–94

R Core Team (2017) R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Ramirez KS, Lauber CL, Knight R, et al (2010) Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91:3463–3470. doi: 10.1890/10-0426.1

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291

Rousk J, Bååth E, Brookes PC, et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–51. doi: 10.1038/ismej.2010.58

Roy-Bolduc A, Laliberté E, Boudreau S, Hijri M (2016) Strong linkage between plant and soil fungal communities along a successional coastal dune system. FEMS Microbiol Ecol fiw156. doi: 10.1093/femsec/fiw156

Rumpel C, Crème A, Ngo P., et al (2015) The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. J soil Sci plant Nutr 15:0–0. doi: 10.4067/S0718-95162015005000034

Schelfhout S, De Schrijver A, De Bolle S, et al (2015) Phosphorus mining for ecological restoration on former agricultural land. Restor Ecol 23:842–851. doi: 10.1111/rec.12264

Schelfhout S, Mertens J, Perring MP, et al (2017) P-removal for restoration of *Nardus* grasslands on former agricultural land: cutting traditions. Restor Ecol 25:S178–S187. doi: 10.1111/rec.12531

Scheurwater I, Cornelissen C, Dictus F, et al (1998) Why do fast- and slow-growing grass species differ so little in their rate of root respiration, considering the large differences in rate of growth and ion uptake? Plant, Cell Environ 21:995–1005. doi: 10.1046/j.1365-3040.1998.00341.x

Schröder JJ, Cordell D, Smit AL, Rosemarin A (2010) Sustainable use of phosphorus, EU Tender ENV.B.1/ETU/2009/0025. Plant Res Int Wageningen

Seguel A, Cumming JR, Klugh-Stewart K, et al (2013) The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza 23:167–83. doi: 10.1007/s00572-013-0479-x

Seinhorst JW (1966) Killing nematodes for taxonomic study with hot f.a. 4 : 1. Nematologica 12:178. doi: 10.1163/187529266X00239

Simons NK, Lewinsohn T, Blüthgen N, et al (2017) Contrasting effects of grassland management modes on species-abundance distributions of multiple groups. Agric Ecosyst Environ 237:143–153. doi: 10.1016/J.AGEE.2016.12.022

Socher SA, Prati D, Boch S, et al (2013) Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. Basic Appl Ecol 14:126–136. doi: 10.1016/J.BAAE.2012.12.003

T’Jollyn F, Bosch H, Demolder H, et al (2009) Ontwikkeling van criteria voor de beoordeling van de lokale staat van instandhouding van de Natura 2000 habitattypen, versie 2.0. Rapp van het Inst voor Natuur- en Bosonderzoek 2009.46:

Tits M, Elsen A, Deckers S, Boon W (2016) Bodemvruchtbaarheid van de akkerbouw- en weilandpercelen in België en noordelijk Frankrijk (2012-2015). Bodemkundige Dienst

Tunlid A, White DC (1991) Biochemical analysis of biomass, community structure, nutritional status and metabolic activity of the microbial communities in soil, , in J. M. Bollag and G. Stotzky (eds). In: Soil Biochemistry. p Vol. 7, pp. 229–262

Van Der Heijden MG a, Bardgett RD, Van Straalen NM (2008) The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. doi: 10.1111/j.1461-0248.2007.01139.x

van der Wal A, Geerts RHEM, Korevaar H, et al (2009) Dissimilar response of plant and soil biota communities to long-term nutrient addition in grasslands. Biol Fertil Soils 45:663–667. doi: 10.1007/s00374-009-0371-1

Van Der Woude BJ, Pegtel DM, Bakker JP (1994) Nutrient limitation after long-term nitrogen fertilizer application in cut grasslands. J Appl Ecol 31:405. doi: 10.2307/2404438

Vuylsteke A, Bergen D, Demuynck E (2014) Schaalgrootte en schaalvergroting in de Vlaamse landen tuinbouw

Wander JGN, van den Berg W, van den Boogert PHJF, et al (2007) A novel technique using the Hendrickx centrifuge for extracting winter sporangia of Synchytrium endobioticum from soil. Eur J Plant Pathol 119:165–174. doi: 10.1007/s10658-007-9156-2

Wardle DA (2002) Communities and ecosystems : Linking the aboveground and belowground components. Princeton University Press

Wardle DA, Bardgett RD, Klironomos JN, et al (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633

White DC, Davis WM, Nickels JS, et al (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40:51–62. doi: 10.1007/BF00388810

Yeates GW, Bongers T, De Goede RG, et al (1993) Feeding habits in soil nematode families and genera-an outline for soil ecologists. J Nematol 25:315–31

TABLES

**Table 1**. Results of the ‘envfit’ analyses. The ‘envfit’ analysis tests for correlations between (co)ordinates of site projections onto the first two co-inertia axes and the soil chemistry variables (i.e. pHKCl, exchangeable Al3+ and Ca2+, total P and POlsen). COIA1 and COIA2 indicate the direction and the strength of the correlation between each soil variable and the first and second axis of COIA, respectively. The goodness-of-fit statistic is the squared correlation coefficient (r²). We assessed significance by a Monte-Carlo test (with 999 random permutations). We found significant correlations with all measured soil variables, thus, only the first three most important variables were shown.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| COIA | Components of COIA | Soil variables | COIA1 | COIA2 | r² | *P-*value |
| Plants - microbiota | Plants | pHKCl | -0.99 | 0.04 | 0.65 | 0.001 |
| POlsen | -0.99 | -0.03 | 0.47 | 0.001 |
| Al+3 | 0.98 | 0.19 | 0.40 | 0.001 |
| Microbiota | pHKCl | -0.99 | 0.02 | 0.67 | 0.001 |
| Al+3 | 0.98 | 0..20 | 0.58 | 0.001 |
| POlsen | -0.98 | 0.20 | 0.22 | 0.02 |
| Plants - nematodes | Plants | pHKCl | 1.0 | -0.002 | 0.64 | 0.001 |
| POlsen | 0.99 | -0.02 | 0.48 | 0.001 |
| Al+3 | -0.99 | -0.004 | 0.35 | 0.003 |
| Nematodes | pHKCl | 0.99 | 0.04 | 0.47 | 0.001 |
| Total P | 0.99 | 0.13 | 0.42 | 0.001 |
| POlsen | 0.99 | 0.15 | 0.28 | 0.007 |
| Microbiota -nematodes | Microbiota | pHKCl | 0.58 | -0.82 | 0.65 | 0.001 |
| Total P | 0.08 | -0.99 | 0.28 | 0.005 |
| Al+3 | -0.69 | 0.72 | 0.57 | 0.001 |
| Nematodes | pHKCl | 0.42 | -0.91 | 0.49 | 0.001 |
| Total P | 0.30 | -0.95 | 0.42 | 0.001 |
| Al+3 | -0.31 | 0.95 | 0.37 | 0.003 |

FIGURE LEGENDS

**Fig. 1**: (a) Clustering of the studied sites into Oligotrophic (blue circles), Mesotrophic (orange triangles) and Eutrophic (red squares) grasslands according to the first two principal components. Soil variables related to soil acidification and fertility were used for this clustering by HCPC (see main text). The centroid of each cluster is represented by a bigger symbol. The percentages in the axes represent the amount of variation explained by each axis. (b-c) Boxplots of the distributions of pHKCl (b) and phosphorus (measured as mg POlsen. kg-1 soil) (c) among the three soil groups. The graph shows that the three clusters were well separated: Oligotrophic soils align with low pH and bioavailable P concentrations but with high exchangeable Al3+ concentrations (Table 1). Eutrophic soils aligned with high pH and bioavailable P concentrations. Mesotrophic soils adopted an overall intermediate position in the principal component analysis.

**Fig. 2**: Co-inertia analysis (COIA) between the plant and microbial community compositions. (a) The positions of each site on the first two axes of the COIA is conditional on the plant (the tail of the arrow) or microbiota (head of the arrow) communities. The colour of the arrows show the site groups according to the HCPC: Oligotrophic sites are in blue, Mesotrophic in orange and Eutrophic in red. The arrow length and angle give the translational coefficient of the site position from one to the other data set. The strength of the correlation between the two datasets for each site is inversely correlated with the length of the arrows. The percentages of variance explained by axes are shown on each axis. (b) Projections of the plant species (see Appendix S2 for complete name) and (c) microbial PLFA functional groups with the highest scores.

**Fig. 3**: Co-inertia analysis (COIA) between the plant and nematode community compositions. (a) The positions of each site on the first two axes of the COIA is conditional on the plant (the tail of the arrow) or nematode (head of the arrow) communities. The colour of the arrows show the site groups according to the HCPC: Oligotrophic sites are in blue, Mesotrophic in orange and Eutrophic in red. The arrow length and angle give the translational coefficient of the site position from one to the other data set. The strength of the correlation between the two datasets for each site is inversely correlated with the length of the arrows. The percentages of variance explained by axes are shown on each axis. (b) Projections of the plant species (see Appendix S2 for complete name) and (c) nematode feeding types with the highest scores.

**Fig. 4**: Co-inertia analysis (COIA) between the microbial and nematode community compositions. (a) The positions of each site onto the first two axes of the COIA is conditional on microbiota (the tail of the arrow) or nematode (head of the arrow) communities. The colour of the arrows show the site groups according to the HCPC: Oligotrophic sites are in blue, Mesotrophic in orange and Eutrophic in red. The arrow length and angle give the translational coefficient of the site position from one to the other data set. The strength of the correlation between the two datasets for each site is inversely correlated with the length of the arrows. The percentages of variance explained by axes are shown on each axis. (b) Projections of the microbial PLFA functional groups and (c) nematode feeding types with the highest scores.

**Fig. 5** Figure showing boxplots of the lengths of arrows in panel (a) in Figs. 2-4 as a function of the different soil groups (i.e. Oligotrophic, Mesotrophic and Eutrophic) and the different co-inertia analyses. Comparisons should be made between different soil groups within each ‘type’ of co-inertia analysis (i.e. plant-microbial, plant-nematode and microbial-nematode COIA).

Appendices

**Appendix S1**: General description of the physical and chemical properties of the studied sites and site-level data.

**Appendix S2**: Overview of plant species found in the study area.

**Appendix S3**: Classification of phospholipid-derived fatty acid markers as indicators of microbiota.

**Appendix S4:** Differences in community compositions and linkages between the three soil group clusters.