

Spatially explicit sampling of AM fungal biomass reveals links to community characteristics and landscape context

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi are important contributors to soil functioning, yet their biomass and diversity may respond differently to environmental characteristics and historical legacies. Understanding these relationships is necessary for evaluating AM fungi as indicators of soil health. We investigated relationships among AM fungal biomass, community properties and natural habitat availability in contemporary and historical agricultural landscapes. AM fungal biomass in the soil was quantified using the fatty-acid biomarker 16:1 ω 5 in phospholipid (PLFA) and neutral lipid (NLFA) fractions. These fractions represent predominantly metabolically active and storage-associated biomass, respectively. Biomass estimates were compared with SSU metabarcoding-derived read count, Shannon diversity, community-weighted niche volume, and culturability. NLFA-based biomass estimates were positively associated with DNA read count and taxon richness but showed weak associations with Shannon diversity, whereas PLFA-based estimates were weakly associated with all three metrics. AM fungal biomass was negatively associated with community-weighted culturability but positively associated with community-weighted niche volume. Neither contemporary nor historical natural habitat availability predicted AM fungal biomass at the spatial scales examined, despite previously identified landscape effects on richness and community composition. Thus, landscape legacies were more evident in AM fungal taxonomic structure than in the amount of fungal biomass maintained in agricultural soils. Our findings demonstrate that biomass, diversity and community ecological characteristics capture distinct dimensions of AM fungal communities and can respond differently to environmental drivers across temporal scales. Integrating lipid-based biomass measurements with molecular assessments of diversity and ecological strategy should therefore provide a more comprehensive basis for evaluating AM fungal community condition and their potential as indicators of soil health in agricultural landscapes.

Keywords: AM fungal biomass, PLFA, NLFA, Culturability, Niche-volume, historical natural habitat availability

1. Introduction

Soil health is defined as the capacity of soil to function as a living ecosystem that sustains plants, animals and humans while maintaining essential ecological processes (Doran and Zeiss, 2000; Kibblewhite et al., 2007; Norris et al., 2020). Unlike traditional concepts such as soil fertility, soil health explicitly integrates biological with physical and chemical components of soil and recognises their interactions in regulating ecosystem functioning (Fierer et al., 2021). Maintaining healthy soils has become a central objective of sustainable agriculture because soil biological processes underpin crop productivity, nutrient cycling, carbon sequestration, water regulation and climate resilience (Romero et al., 2023). Consequently, biological indicators have become increasingly important for evaluating soil condition alongside conventional chemical and physical measurements (Bender et al., 2016).

Among soil organisms, arbuscular mycorrhizal (AM) fungi (subphylum *Glomeromycotina*) are particularly important indicators of soil health because they form mutualistic associations with approximately 80% of terrestrial plant species, including most agricultural crops (van der Heyde et al., 2017; Lehman et al., 2019). Through extensive extraradical hyphal networks, AM fungi exchange plant-derived carbon for mineral nutrients, particularly phosphorus and nitrogen, thereby enhancing plant productivity while simultaneously improving soil aggregation, increasing carbon storage and reducing nutrient losses (Powell and Rillig, 2018; Wu et al., 2024; Liu et al., 2025). In addition, AM fungi enhance plant tolerance to abiotic stress, suppress soil-borne pathogens and influence plant community dynamics, making them fundamental contributors to both ecosystem resilience and agricultural sustainability (Delavaux et al., 2017; Tang et al., 2024). Because AM fungal communities respond to environmental change and agricultural disturbance, while potentially retaining legacies of past landscape conditions, they can provide sensitive indicators of both contemporary and historical influences on soil condition and management impacts (Moora et al., 2014; Rillig et al., 2019).

AM fungal responses to environmental change can be characterised through complementary measures of biomass and community composition, yet these components have rarely been evaluated in parallel. Biomass provides information on the quantity of fungal structures present in soil, whereas DNA metabarcoding characterises taxonomic diversity and composition and can further resolve ecological characteristics of constituent taxa, including their realised niche characteristics and culturability (i.e., whether phylogroups correspond to AM fungal species held in culture collections (Ohsowski et al., 2014)). Integrating these approaches may therefore provide a more complete assessment of how AM fungal communities respond to environmental change than either approach alone.

The functional contribution of AM fungi to plant performance depends on their biomass and community composition (Fierer et al., 2021; Stewart et al., 2026). Extraradical hyphae represent the principal interface through which AM fungi acquire nutrients and contribute to soil aggregation, making fungal biomass in soil an important measure of ecosystem functioning (Powell and Rillig, 2018; Olsson and Lekberg, 2022). Agricultural intensification frequently reduces AM fungal biomass through repeated tillage, high phosphorus fertilisation and simplified crop rotations, all of which disrupt hyphal networks or reduce plant investment in the symbiosis (Olsson et al., 1997; van Groenigen et al., 2010; Bender et al., 2016; Vahter et al., 2025). Soil microbial biomass is commonly estimated using lipid biomarkers (van Galen et al., 2025). Studies of AM fungal biomass typically target the fatty acid 16:1 ω 5, which occurs in phospholipid fatty acids (PLFAs) representing metabolically active hyphae and neutral lipid fatty acids (NLFAs) that largely reflect storage structures such as spores and vesicles (Olsson, 1999; Olsson and Johansen, 2000; Olsson and Lekberg, 2022). Increasingly, these biomass estimates are complemented by DNA metabarcoding, where the number of recorded AM fungal reads per sample is sometimes used as an indicator of fungal biomass, given the samples were of equal weight and the DNA concentrations were not standardised among samples during any of the interim steps (Moora et al., 2016). However, the extent to which AM fungal read abundance corresponds to independently measured lipid biomass remains poorly resolved. In particular, differences in the biological structures represented by molecular and lipid-based measures may influence the strength of this relationship.

AM fungal biomass may also be linked to community diversity. A substantial body of evidence indicates that diversity and biomass of soil microbiota, including fungi, exhibit a unimodal relationship across broad environmental gradients (Geyer and Barrett, 2019; Bastida et al., 2021). This relationship is generally positive under resource-limited conditions but may become negative at high biomass or resource availability, potentially owing to increasing competitive exclusion (Geyer and Barrett, 2019; Bastida et al., 2021). Among AM fungi, experimentally increasing species richness can enhance the length of mycorrhizal hyphae in soil, providing evidence of a direct link between diversity and fungal biomass (van der Heijden et al., 1998). However, whether variation in AM fungal diversity is associated with their biomass in field settings, including agricultural landscapes, remains poorly understood.

Besides taxonomic diversity, metabarcoding can reveal ecological characteristics of AM fungal communities that may help explain variation in biomass. Recent approaches to metabarcoding data analysis have enabled the estimation of niche characteristics, including multidimensional niche volumes and host-resource preferences, for molecular taxa (such as the virtual taxa (VT); Öpik et al., 2010), facilitating the use of trait-based approaches in AM fungal ecology (Davison et al., 2024). Likewise, AM fungal taxa differ in their culturability, which has been proposed as a proxy for a ruderal life-

history strategy (van der Heijden et al., 2008), with culturable taxa generally exhibiting greater tolerance to disturbance than unculturable taxa (Ohsowski et al., 2014). Agricultural intensification has been shown to favour disturbance-tolerant generalists while reducing specialist taxa with narrower ecological niches, resulting in functional homogenisation of AM fungal communities even when taxonomic richness remains relatively high (Peng et al., 2024; Vahter et al., 2025). Recent evidence from a range of habitats further suggests that communities dominated by narrower-niched and less culturable taxa may support greater AM fungal biomass (Vahter et al., 2025), but whether these community characteristics consistently covary with independent lipid-based measures of biomass within an individual landscape type remains unresolved.

The distribution of AM fungi is spatially structured. At broader landscape scales, semi-natural habitats such as grasslands and natural habitats such as forests act as reservoirs of AM fungal propagules and sources of colonisation for surrounding agricultural fields (Mennicken et al., 2020; Vahter et al., 2022). Consequently, fields embedded within heterogeneous landscapes generally support greater AM fungal diversity and biomass than those surrounded by simplified agricultural matrices (Boeraeve et al., 2019; Mennicken et al., 2020). Despite extensive work on biomass responses to field management, considerably less is known about how natural habitat availability influences biomass across spatial scales.

In addition to contemporary landscape structure, historical land use may exert long-lasting influences on soil biological communities. Legacy effects arising from former agricultural practices or historical habitat loss can persist for decades or centuries by altering soil physicochemical properties, propagule availability and dispersal processes (Fichtner et al., 2014; Liu et al., 2023; Zárate Martínez et al., 2024; Vahter et al., 2026). Recent work has demonstrated that historical natural habitat availability explains contemporary patterns of AM fungal diversity better than present-day landscapes in some agricultural systems (Zárate Martínez et al., 2024). Moreover, studies have reported positive relationships between AM fungal diversity and extraradical biomass, suggesting that the loss of specialist taxa following agricultural intensification may reduce not only species richness but also the functional capacity of AM fungal communities (Vahter et al., 2025). Whether these landscape legacies extend to AM fungal biomass and community functional characteristics, however, remains unknown. Determining whether biomass responds to natural habitat availability over similar spatial and temporal scales as diversity and community composition would reveal whether historical landscape change leaves parallel legacies in the amount and composition of AM fungi.

Together, these complementary measures provide an opportunity to determine whether landscape-driven changes in AM fungal community composition are accompanied by corresponding changes in fungal biomass and functional characteristics. Addressing

these gaps is important for identifying the landscape contexts that best support functionally diverse and productive AM fungal communities. We therefore evaluated how natural habitat availability at multiple spatial and temporal scales influences contemporary AM fungal biomass and examined relationships among fatty-acid-based biomass, molecular read counts, taxonomic diversity, and community characteristics. We tested the following hypotheses: (1) AM fungal biomass is positively associated with DNA read counts, but the strength of the relationship may differ between NLFAs and PLFAs if the different components of AM fungal biomass contribute differently to DNA-based abundance; (2) AM fungal biomass is positively associated with AM fungal richness, as agricultural fields are expected to fall on the low-biomass / low-richness portion of a hump-shaped AM fungal richness-biomass relationship; (3) AM fungal biomass is negatively associated with community-weighted mean niche volume and culturability, consistent with previous evidence linking greater biomass to narrower niche characteristics and lower culturability; and (4) spatial and temporal patterns in natural habitat availability predict AM fungal biomass at scales (spatial scales: 50, 100 and 200 m; map years: 1894, 1969, and 2022) similar to those previously identified for AM fungal diversity and community composition.

2. Methods

Study sites, experimental design, and sampling

The study was carried out in three organically managed wheat (*Triticum aestivum* L.) fields in Tartu County, Estonia (Fig. 1). In the year of sampling (Sept. 2021), wheat was intercropped with white clover (*Trifolium repens* L.), and the soil samples were collected after the harvest and prior to soil tillage or fertilisation. The fields were located within 50 km of each other and experienced very similar climatic conditions.

Sample collection was described previously in Zarate-Martinez et al. (2024). Briefly, a total of 99 samples were collected following a 100 × 100 m North-South oriented grid with sampling (from the top 10 cm) at the intersection of gridlines. Soil was sampled in order to conduct molecular, fatty acid and chemical analysis. For the molecular analysis, approximately 10 g of soil was packed in permeable paper bags and placed in grip-seal bags with silica gel for desiccation. Additionally, for soil chemical properties and soil fatty acid analysis, approximately 500 g of soil was collected and air-dried at room temperature.

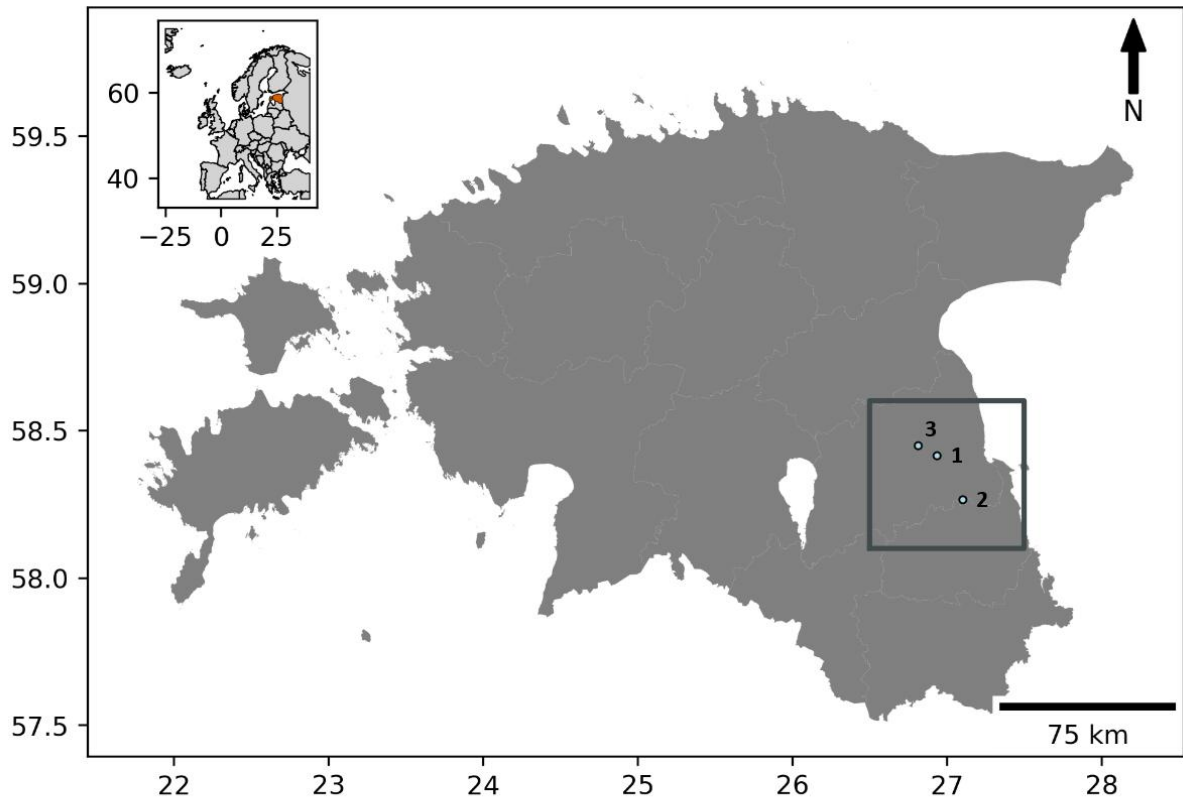


Fig. 1. Blue dots represent the location of sampled fields in Southeast Estonia: Site 1, Site 2, Site 3. The intersections of gridlines (sampled points) following a 100×100 m North-South oriented grid are represented by black points. The map was made using freely available vector and raster map data from Natural Earth ([naturalearthdata.com](https://www.naturalearthdata.com)) and the Estonian Administrative and Settlement Division: Land Board; accessed 07.05.2026.

Current and historical availability of natural habitat surrounding each sampling location was quantified using topographic maps obtained from the Republic of Estonia Land and Spatial Development Board geoportal (geoportaal.maaamet.ee, accessed 2023). Three map series representing different time periods were used, corresponding to the map years 1894 (1:42 000), 1969 (1:25 000), and 2022 (1:10 000). An unavoidable consequence of using maps with different spatial scales was variation in the precision of estimated natural habitat availability. To identify the spatial and temporal scales most strongly associated with AM fungal community, we adopted a multiscale approach.

Following Zarate-Martinez et al. (2024), natural habitat availability was estimated as the proportion of forest and meadow habitat relative to agricultural land within circular buffers of 50, 100 and 200 m around each sampling point for each map year. A temporal decline in natural habitat availability was evident across all three fields over the three historical periods examined (Fig. S1).

DNA metabarcoding and bioinformatics

DNA metabarcoding and the bioinformatic analysis were conducted and described in Zarate-Martinez et al. (2024). Briefly, environmental DNA was extracted from 5 g of air-dried soil using the PowerMax® Soil DNA Isolation Kit with a modified extraction protocol following Gazol et al. (2016). Arbuscular mycorrhizal (AM) fungal DNA was amplified using the SSU rRNA gene primers WANDA (Dumbrell et al., 2011) and AML2 (Lee et al., 2008) in a two-step PCR protocol incorporating Illumina Nextera XT adapters and dual indices. Purified amplicons were sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end sequencing; at Asper Biogene, Tartu, Estonia).

Sequence processing was performed using the gDAT pipeline (Vasar et al., 2021). Reads were quality-filtered, merged with FLASH (Magoč and Salzberg, 2011), and screened for chimeras using VSEARCH against the MaarjAM database (Rognes et al., 2016; Öpik et al., 2010). Virtual taxa (VT) were assigned using BLAST+ (Camacho et al., 2009) against the MaarjAM database with 97% sequence identity and 95% alignment. Raw sequencing data are available in the NCBI Sequence Read Archive under BioProject PRJNA1122197. A total of 836,708 sequences corresponding to 122 VT were recovered from the 99 soil samples. Samples containing fewer than 100 AM fungal reads were designated for exclusion from subsequent analyses; however, all samples exceeded this threshold. VT represented by a single read (singletons) were excluded from subsequent analyses. The cleaned data consisted of 836 702 reads of 116 VTs in the 99 samples.

Soil chemical analysis

Soil chemical analysis was conducted and described in Zarate-Martinez et al. (2024). Soil chemical properties were analysed at the Estonian University of Life Sciences (Tartu). Soil-available phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) concentrations were determined using Mehlich III extraction followed by inductively coupled plasma optical emission spectrometry (ICP-OES). Organic carbon concentration was determined using a sulfochromic oxidation and spectrophotometric detection, while soil pH was determined from a 1 M KCl solution.

To account for correlations among measured soil variables, principal component analysis (PCA) was performed on soil pH, organic carbon, P, K, Ca and Mg after log transforming and standardising all variables to zero mean and unit variance. The first two principal components, explaining 55.7% and 20.7% of the total variation, respectively (Fig. S2), were included as covariates in subsequent models. PC1 was

primarily associated with soil pH, Ca and Mg, whereas PC2 was mainly associated with organic carbon, P and K.

Soil fatty acid analysis

In the current study, soil fatty acid analysis was conducted on the same soil samples. Soil phospholipid (PLFA) and neutral lipid (NLFA) fatty acid analysis followed an established protocol (van Aarle and Olsson, 2003), adapted for 96-well plate processing (Buyer and Sasser, 2012). Lipids were extracted from approximately 2 g of dry soil sample using a monophasic mixture of chloroform, methanol, and citrate buffer. Following centrifugation, the supernatant was split into two phases by adding additional chloroform and citrate buffer.

The lower lipid-rich phase was collected and dried under a stream of nitrogen at 40 °C. Lipids were subsequently fractionated on silica 96-well solid-phase extraction plates into neutral, intermediate, and phospholipid fractions using chloroform, acetone, and methanol as eluents, respectively. The phospholipid and neutral lipid fractions were dried, after which nonadecanoic acid methyl ester (Me19:0) was added as an internal standard. Fatty acids were converted to fatty acid methyl esters by mild alkaline methanolysis using potassium hydroxide in methanol: toluene at 37 °C, and the reaction was halted with acetic acid. Following phase separation with chloroform, the lipid fraction (lower phase) was collected and dried under nitrogen.

Fatty acid methyl esters were resuspended in toluene and analysed by gas chromatography with flame ionisation detection (FID) following Frostegard et al. (1993). Fatty acid concentrations (nmol g⁻¹ of dry soil) were quantified relative to the internal standard. PLFA 16:1 ω 5 and NLFA 16:1 ω 5 were used as indicators of arbuscular mycorrhizal fungal biomass (Olsson, 1999; Olsson and Johansen, 2000).

AMF community characterisation

Realised niche hypervolumes for each virtual taxon (VT) were obtained from Davison et al. (2024). In that study, niche characteristics were estimated by integrating global metabarcoding data on AM fungal VT occurrence with corresponding measurements of abiotic and biotic environmental variables collected from the same soil samples. The full niche volume takes into account the total hypervolume occupied by a taxon within a multidimensional niche space represented by measured abiotic (pH, mean annual temperature, organic soil carbon, Ca, K, Mg, P, mean annual precipitation) and biotic (fungal, plant, prokaryote composition and host plant abundance) environmental gradients (Davison et al., 2024). Taxon culturability was used as an indicator of putatively ruderal AM fungal taxa following Ohsowski et al. (2014).

Statistical analyses

AM fungal richness (Hill number of order $q = 0$) was obtained from Zárate-Martínez et al. (2024), while Shannon diversity (Hill number of order $q = 1$) was additionally calculated to incorporate differences in taxon relative abundances. Hereafter, these indices are referred to as richness and Shannon diversity, respectively. In contrast to richness, which weights all taxa equally, Shannon diversity provides an abundance-sensitive diversity measure that may be more directly comparable with biomass estimates, which inherently reflect differences in taxon abundance. Both diversity metrics were estimated using asymptotic extrapolation to complete sample coverage with the iNEXT() function in the R package iNEXT (version 3.0.2; Chao et al., 2014; Hsieh et al., 2025). Estimated richness values were rounded to the nearest integer before analysis. AM fungal PLFA- and NLFA-based biomass concentrations were transformed using the natural logarithm of $(x + 1)$, upon assessment for normality, prior to analysis to reduce right-skewness. For each soil community, community-weighted mean (CWM) values of niche volume were calculated by weighting the niche hypervolume of individual taxa according to their proportional sequence abundance within the community. Thus, each CWM represents the average niche attribute of the AM fungal assemblage while accounting for the relative abundance of its constituent taxa. The CWM realised niche volume was transformed using the natural logarithm to satisfy model assumptions. Community-level culturability was calculated as a community-weighted mean based on the proportional abundance of sequences assigned to each taxon. CWM culturability was logit-transformed using the car package in R (Fox et al., 2023) prior to modelling. Pearson correlation analyses were performed to assess the relationships between AM fungal biomass and DNA read count, richness, Shannon diversity, CWM niche volume, and CWM culturability.

To identify the spatial and temporal scales at which natural habitat availability best explained AM fungal alpha diversity index (Shannon diversity), biomass (AM fungal PLFA and NLFA), CWM niche volume and CWM culturability, separate models were constructed for each combination of spatial scale (50, 100 and 200 m) and historical time period (years 1894, 1969, and 2022). Habitat proportions were logit-transformed prior to modelling. The models for richness and beta diversity indices (turnover and nestedness) have been previously described in Zárate-Martínez et al. (2024). Because habitat availability measured at different spatial scales is inherently correlated, these variables were evaluated in separate models rather than within a single model (Riibak et al., 2024). Consequently, nine candidate models (three spatial scales $\times$ three temporal scales) were fitted for each analysed metric, each including natural habitat availability together with soil chemistry (PC1 and PC2) as explanatory variables. Correlations among explanatory variables remained below 0.5 in the buffer radii 50 m and 100 m (Fig. S3, Fig. S4). In the buffer radius 200 m, the natural habitat proportion in 1894, 1969 and 2022 showed a moderate pairwise correlation (maximum $r = 0.62$) (Fig. S5), indicating

some shared variation but no evidence of severe multicollinearity. All statistical analyses were conducted in R version 4.6.1 (R Core Team, 2026).

Relationships between diversity indices, biomass, community characteristics, and natural habitat availability were examined using linear models (LMs). Models were initially fitted both with and without field identity as a random effect. Field identity was excluded from final models as initial random-intercept variance was negligible, matching analytical approach of Zárate-Martínez et al. (2024). Models were compared by using the Akaike Information Criterion (AIC). Candidate models with $\Delta AIC < 2$ were considered to have the best fit (Guisan and Zimmermann, 2000) and were used to identify the spatial and temporal scales at which natural habitat availability exerted the strongest influence. Model performance was evaluated using R^2 .

Potential spatial autocorrelation was assessed by calculating Moran's I on model residuals using geographic distance matrices and the Moran.I function from the ape package (Paradis et al., 2004). No significant spatial autocorrelation was detected for any fitted model. Partial regression plots were generated using the visreg package (version 3.0.0; Breheny and Burchett, 2017).

3. Results

Communities

The cleaned data consisted of virtual taxa (116 AMF VTs) from nine families, of which Glomeraceae was the most abundant (52.6%) followed by Claroideoglomeraceae (20.1%) and Paraglomeraceae (9.8%) (Fig. S6). AM fungal richness ranged between 17 and 81 virtual taxa (VT), averaging 42 VT per sample and the Shannon diversity was between 3.36 and 21.74, averaging 11.02. Here, a total of 35 AM fungal VT were classified as putatively culturable, whereas 81 were considered unculturable. However, sequence read abundance was dominated by the culturable taxa, which collectively accounted for more reads than the unculturable VT (Fig. S7).

Correlations among community characteristics

PLFA- and NLFA-based estimates of AM fungal biomass exhibited considerable variation within fields (Figs. 2 and 3). PLFA- and NLFA-based AM fungal biomass measurements ranged from 0.001 to 5.78 and from 0.001 to 14.03 nmol g⁻¹ dry soil, respectively.

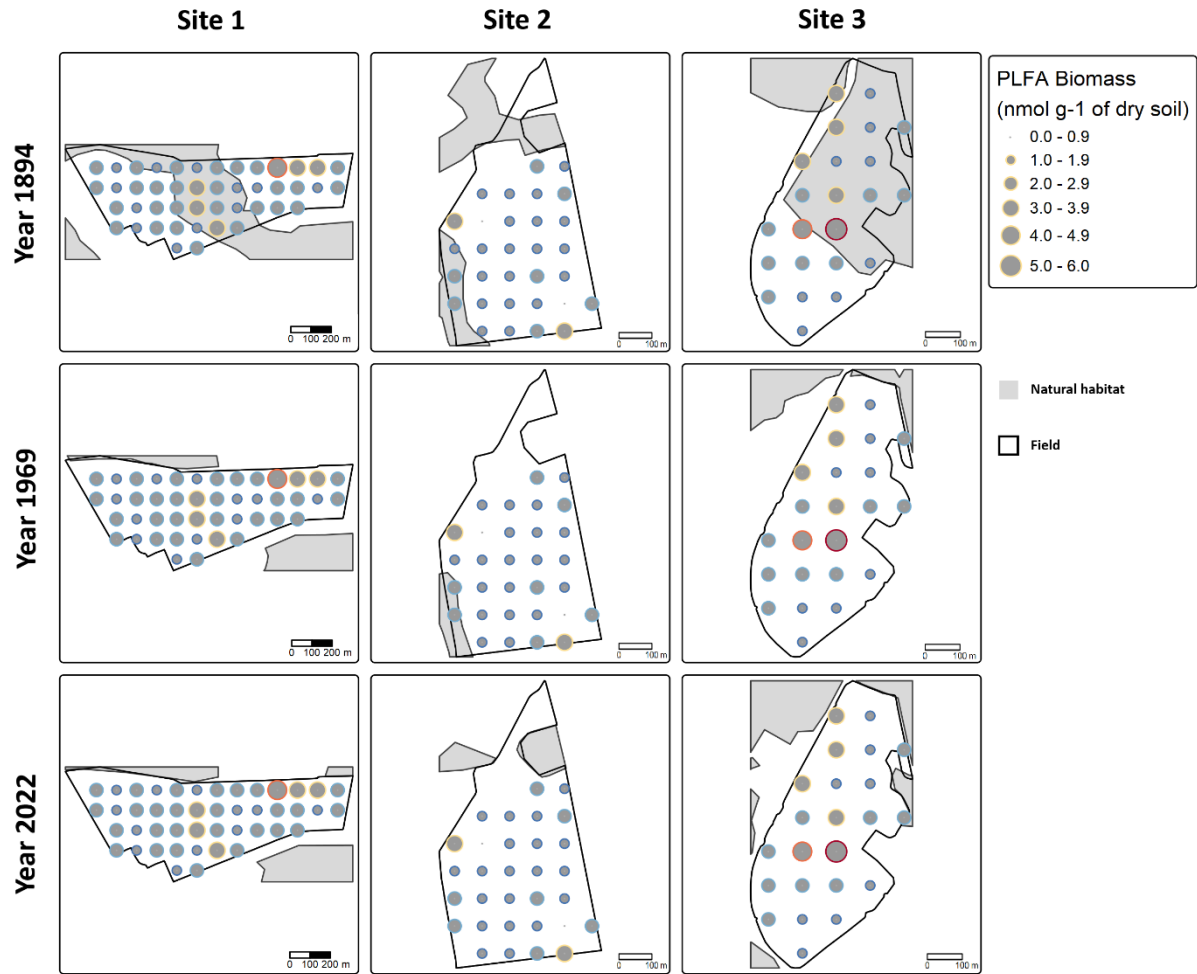


Fig. 2. AM fungal PLFA-based biomass at the three study sites showing availability of natural habitat at each historical map year (1894, 1969, and 2022).

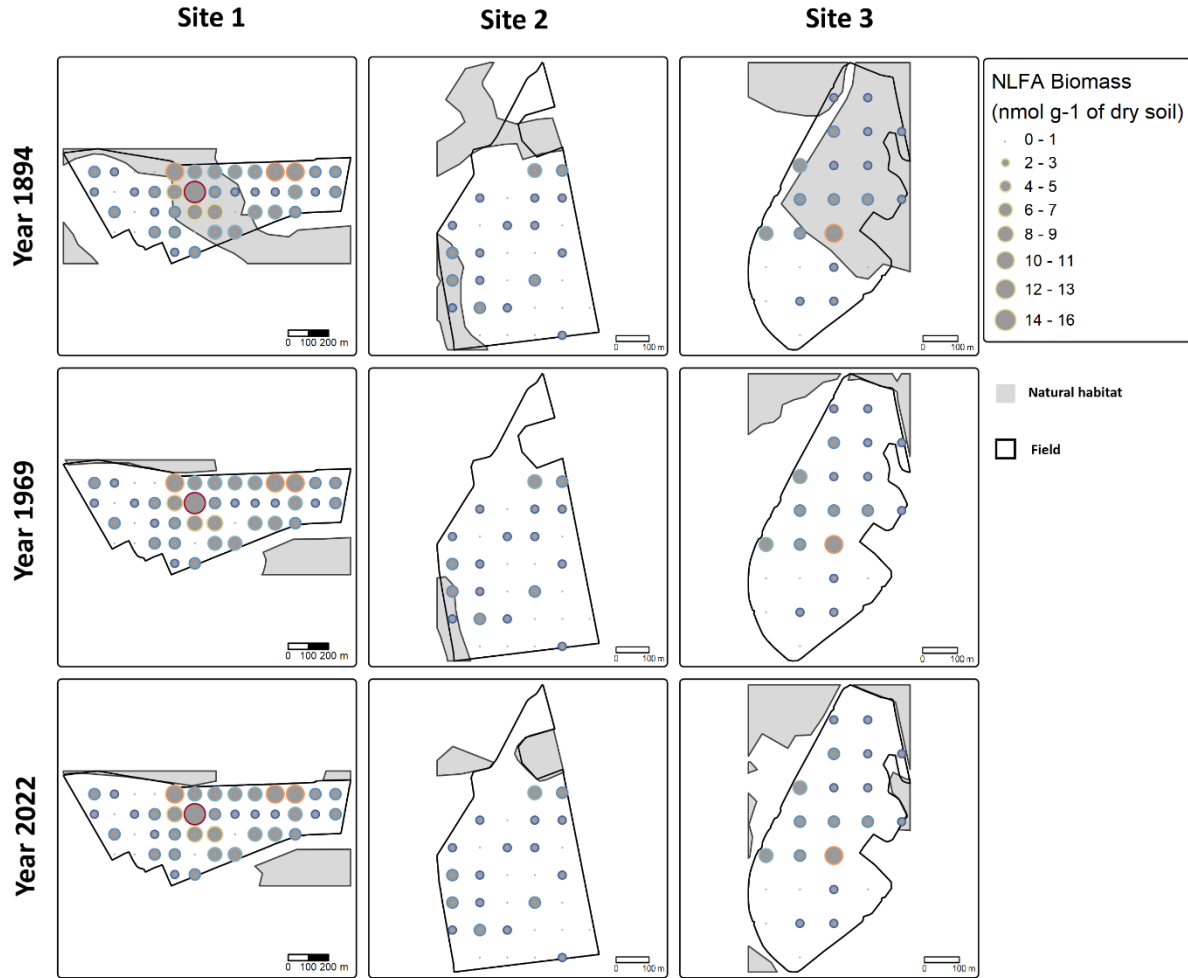


Fig. 3. AM fungal NLFA-based biomass at the three study sites showing availability of natural habitat at each historical map year (1894, 1969, and 2022).

PLFA- and NLFA-based estimates of AM fungal biomass were moderately positively correlated with each other ($r = 0.53$, $p < 0.001$; Fig. 4). NLFA also exhibited moderate positive correlations with DNA read count ($r = 0.28$, $p = 0.005$) and richness ($r = 0.39$, $p < 0.001$), while its relationship with Shannon diversity was weak ($r = 0.14$, $p = 0.180$; Fig. 4). The PLFA biomarker showed only weak positive associations with DNA read count, richness, and Shannon diversity ($r = 0.06$, 0.16 , and 0.11 ; $p = 0.556$, 0.121 , and 0.264 , respectively; Fig. 4).



Fig. 4. Correlation (Pearson) of alpha diversity indices and the AM fungal biomass indicators. Values in the lower triangle are correlation coefficients (r), while stars in the upper triangle indicate significance: * - $P < 0.05$, ** - $P < 0.01$, and *** - $P < 0.001$.

Both PLFA- and NLFA-based AM fungal biomass estimates showed moderately strong positive correlations with the community-weighted mean (CWM) realised niche volume ($r = 0.63$ and 0.54 , respectively; both $p < 0.001$; Fig. 5) and moderate negative associations with CWM culturability (PLFA: $r = -0.32$, $p = 0.001$; NLFA: $r = -0.33$, $p < 0.001$; Fig. 5). CWM culturability was also negatively correlated with CWM realised niche volume ($r = -0.54$, $p < 0.001$; Fig. 5).

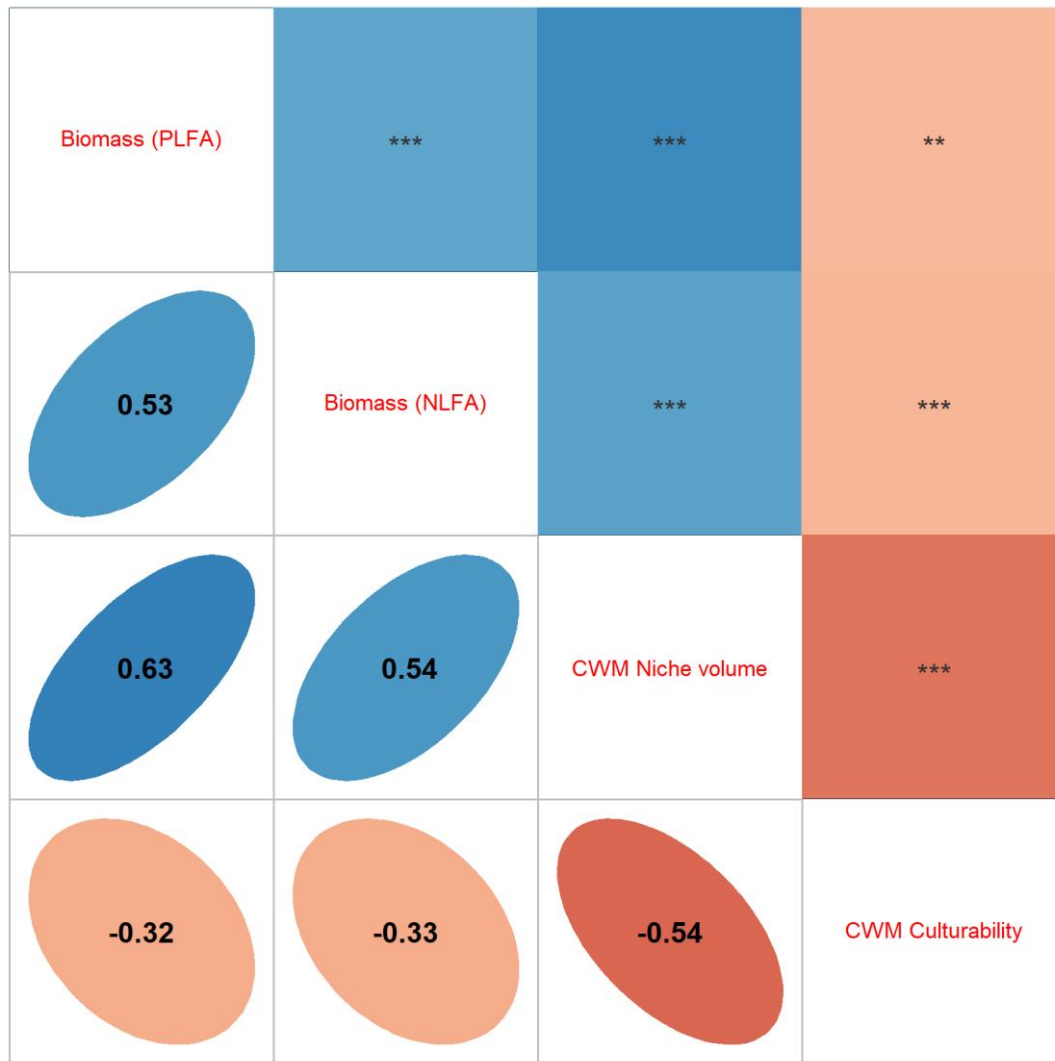


Fig. 5. Correlation (Pearson) of AMF niche volume (CWM), AMF culturability (CWM) and the AM fungal biomass indicators. Values in the lower triangle are correlation coefficients (r), while stars in the upper triangle indicate significance: * - $P < 0.05$, ** - $P < 0.01$, and *** - $P < 0.001$.

Availability of natural habitats

All combinations of spatio-temporal scales for models of PLFA- and NLFA-based AM fungal biomass had ΔAIC values < 2 (Fig. 6, Table S Excel), indicating similar model support. The spatial distributions of PLFA- and NLFA-based AM fungal biomass across the three study sites, together with natural habitat availability in 1894, 1969, and 2022, are shown in Figs. 2 and 3, respectively. While the best supported models (i.e., those with the lowest AIC) included recent past (year 1969) natural habitat availability (at medium and larger spatial scales for PLFA- and NLFA-based biomass estimates, respectively), neither PLFA- nor NLFA-based AM fungal biomass was significantly related to the historical availability of natural habitats. Rather, soil chemistry, in the form of PC1 and PC2 explained the observed variation for PLFA-based biomass, and PC1 solely explained the variation for NLFA-based biomass (Fig. 6, Table S Excel). Overall, there was

no significant relationship with the historical availability of natural habitats on the PLFA- and NLFA-based AM fungal biomass estimates.

The analysis of spatio-temporal scales and the asymptotic AM fungal Shannon diversity index produced three models with $\Delta AIC < 2$ (Fig. 6, Table S Excel). Of the three models, the top two showed a significant positive relation with the past (year 1894) natural habitat availability at 200 m and 100 m buffer radii, respectively (Fig. 6, Table S Excel). In all candidate models, the PC1 and PC2 were not significant predictors (Fig. 6, Table S Excel).

Multiple candidate models received comparable support ($\Delta AIC < 2$) for both CWM AM fungal realised niche volume (six of nine models) and the CWM of culturable AM fungal taxa (five models; Fig. 6, Table S Excel). Neither response showed a significant association with spatio-temporal variation of natural habitat availability, whereas PC1 was a consistently significant predictor across all supported models ($p < 0.05$; Fig. 6, Table S Excel), mirroring the pattern observed for NLFA-based AM fungal biomass.

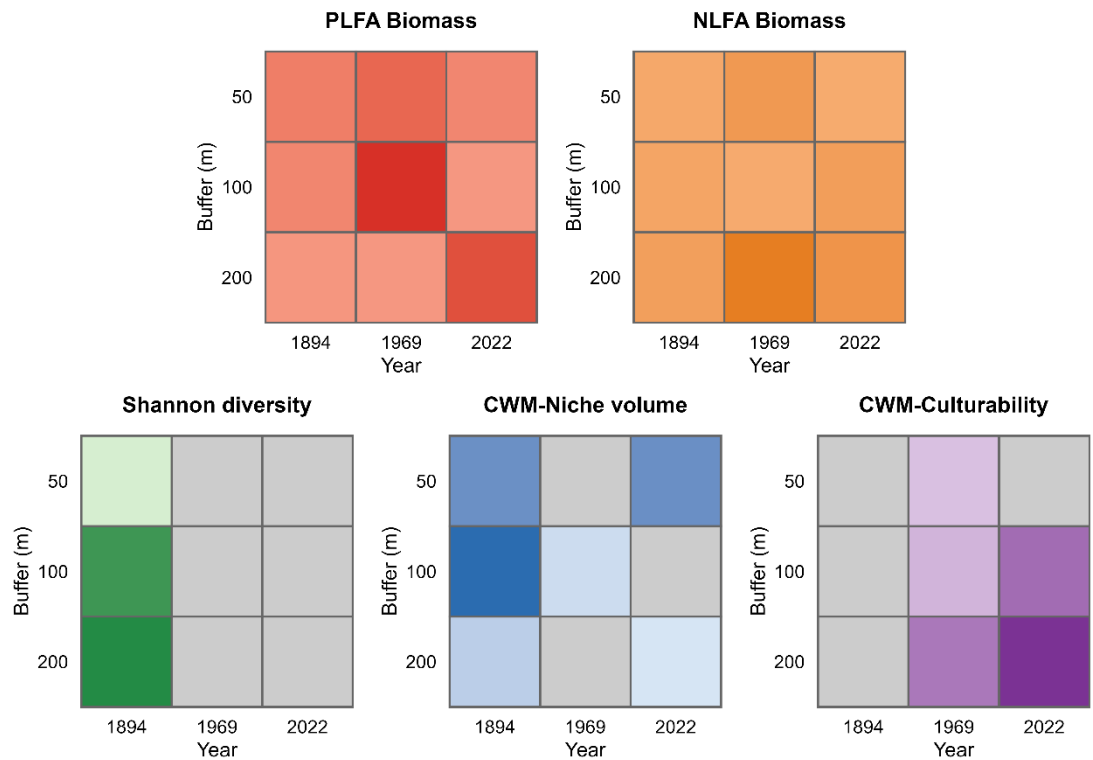


Fig. 6. Model support for the effects of spatial and temporal scales on arbuscular mycorrhizal (AM) fungal community properties. Grids represent models combining three spatial scales (50, 100, and 200 m buffer radii) and three temporal scales (map years 1894, 1969 and 2022) for PLFA biomass, NLFA biomass, Shannon diversity, community-weighted mean (CWM) niche volume, and CWM culturability. Coloured cells indicate

models with $\Delta AIC < 2$, with darker colours representing lower ΔAIC values, whereas grey cells indicate models with $\Delta AIC \geq 2$.

4. Discussion

Biomass covaries with DNA read count and richness

NLFA-based AM fungal biomass estimates were positively associated with SSU sequence read counts and AM fungal taxon richness, but only weakly with Shannon diversity. Therefore, biomass appears to reflect the size and taxonomic breadth of the AM fungal community more closely than a Shannon index integrating both richness and evenness. The positive biomass-richness relationship is consistent with evidence that agricultural intensification can reduce both AM fungal richness and biomass (Vahter et al., 2025). Shared responses to resource availability, plant carbon allocation, nutrient enrichment, and soil pH may contribute to this coupling, because these factors can influence both which taxa persist and the biomass they maintain (Treseder, 2004; Grilli et al., 2013; Moora et al., 2014; Qin et al., 2015; Roy et al., 2017; Williams et al., 2017; Boeraeve et al., 2019).

The stronger relationship between richness and NLFA 16:1 ω 5 than between richness and PLFA 16:1 ω 5 suggests that richness was more closely associated with accumulated fungal biomass than with the metabolically active mycelium in soil. PLFA 16:1 ω 5 is primarily associated with membrane lipids, whereas NLFA 16:1 ω 5 is more strongly associated with storage structures such as spores and vesicles (Hedlund, 2002; Qin et al., 2015; Turgay et al., 2015). Because AM fungi vary in biomass allocation among intraradical, extraradical, and storage structures (Olsson et al., 1997; Olsson and Johansen, 2000; Stevens et al., 2020), the two lipid fractions need not correspond identically with community composition. Furthermore, because PLFA 16:1 ω 5 may also be present in some Gram-negative bacteria, it provides a less specific estimate of AM fungal biomass than NLFA 16:1 ω 5, which is considered a more sensitive biomarker of AM fungal biomass (Lekberg et al., 2022; Olsson and Lekberg, 2022). Overall, these results indicate that richness and biomass describe related but distinct aspects of AM fungal communities.

Culturability is negatively associated with biomass

AM fungal biomass was negatively associated with AM fungal culturability in both NLFA and PLFA fractions. This pattern is consistent with previously reported differences in life-history strategy among AM fungal taxa (Vahter et al., 2025). Culturable taxa are disproportionately represented by disturbance-tolerant generalists, particularly within Glomeraceae and Diversisporaceae, whereas many less readily cultured Gigasporaceae and Acaulosporaceae are associated with more specialised strategies (Ohsowski et al., 2014; Vahter et al., 2025). Differences in biomass allocation may contribute to this pattern, if, as proposed, culturable taxa allocate relatively more biomass to intraradical

structures, whereas non-culturable taxa invest more in extraradical mycelium (Chagnon et al., 2013; van der Heyde et al., 2017). The negative relationships across both lipid fractions suggest that greater representation of culturable taxa may be associated with smaller pools of both active hyphal and storage/reproductive biomass. However, because culturability is closely linked to taxonomic identity and ecological strategy, it is more appropriately interpreted as a community-level indicator of life-history composition than as a direct driver of biomass. The result is nevertheless consistent with evidence that agricultural intensification can increase the representation of culturable taxa while reducing total AM fungal biomass (Vahter et al., 2025).

Biomass is positively associated with niche volume, but the relationship is context-dependent

Biomass was positively associated with community-weighted niche volume, contrasting with the negative relationship reported under intensive agricultural management (Vahter et al., 2025). This contrast suggests that AM fungal CWM niche volume does not have a consistent relationship with AM fungal biomass, but depends on the characteristics of the taxa contributing to community-level niche structure and the environmental context. It is notable that this study incorporated a short environmental gradient (organic arable fields within a small spatial region), compared with Vahter et al. (2025), which incorporated multiple habitat types over a larger spatial extent. It is possible that the niche volume-biomass relationship differs within, compared to between, habitats or that it forms a non-linear relationship, of which this study represents only a particular fraction. Under intensive management, increased community-weighted niche volume has been linked to the persistence of disturbance-tolerant generalists following the loss of specialist taxa (Vahter et al., 2025). AM fungal niche characteristics are also phylogenetically structured, with several Glomeraceae lineages tending towards broader niches than Acaulosporaceae and Gigasporaceae (Davison et al., 2021; Vahter et al., 2025). In our system, however, broad niche occupancy was not associated with reduced biomass. Instead, it may have reflected taxa capable of exploiting heterogeneous resources or environmental conditions while maintaining substantial biomass. AM fungal niches are shaped jointly by abiotic filters and resource availability, including soil pH, temperature, host abundance, and nutrients (Davison et al., 2021, 2024), while biomass allocation varies with host association and soil resource availability (Stevens et al., 2020). Thus, the contrasting biomass-niche relationships suggest that niche breadth alone is insufficient to predict biomass; the identity, life-history strategy, and resource context of the taxa contributing to niche volume are also important (Chagnon et al., 2013).

Landscape legacies persist in AM fungal richness but not biomass

In contrast to AM fungal richness (Zárate Martínez et al., 2024), neither contemporary nor historical natural habitat availability significantly predicted AM fungal biomass, at

any of the spatial scales assessed. Instead, our results indicate that landscape structure leaves a stronger signal in AM fungal richness than in the amount of fungal biomass maintained in agricultural soils. This divergence is notable because natural and semi-natural habitats surrounding agricultural fields can influence AM fungal communities through host availability and propagule spill-over, with effects on community composition and richness detected at scales of approximately 100 m (Pirhofer Walzl et al., 2022; Zárate Martínez et al., 2024). Landscape composition can also explain variation in soil microbial biomass more generally (Culman et al., 2010). Nevertheless, AM fungal biomass can be highly heterogeneous at fine spatial scales and partly decoupled from taxonomic richness because local biomass may be disproportionately determined by individual mycelia (Mummey and Rillig, 2008; Koorem et al., 2014). Such local heterogeneity may weaken landscape-level signals in biomass even where landscape structure influences the occurrence and persistence of AM fungal taxa.

The contrast was particularly evident for historical landscape structure: historical natural habitat availability was associated with AM fungal richness and species turnover, consistent with persistent landscape legacies (Zárate Martínez et al., 2024), but not with either measure of biomass. Importantly, this divergence occurred despite the positive relationship between AM fungal richness and biomass observed in our study. Thus, although communities containing more taxa tended to support greater biomass, the two community properties did not respond equivalently to landscape history. This suggests that the processes generating variation in richness and biomass are at least partly distinct.

Historical habitat availability may primarily determine which AM fungal taxa persist in agricultural landscapes through long-term effects on colonisation, dispersal and local extinction, whereas the biomass maintained by those communities may respond more strongly to contemporary environmental conditions. Host plants, soil fertility, nutrient availability and agricultural management can directly regulate AM fungal growth and carbon allocation, potentially overriding or obscuring historical landscape effects on biomass (Cobb and Wilson, 2018; Vahter et al., 2025). Changes in biomass following land-use transitions may also depend on the direction and duration of land-use change: conversion of natural grasslands to cropland can reduce AM fungal biomass, with potentially slow or compartment-specific recovery (Cobb and Wilson, 2018), whereas increased organic inputs following afforestation can promote microbial biomass recovery (Zhang et al., 2016). Together, these findings suggest that landscape legacies are more persistent in the taxonomic structure of AM fungal communities than in their contemporary biomass. Historical habitat loss may therefore leave a detectable biodiversity signal even after its influence on the total fungal biomass pool has weakened or become obscured by contemporary environmental conditions.

Biomass provides complementary information to taxonomic richness for assessing soil health

Our results demonstrate that AM fungal biomass and taxonomic diversity capture complementary dimensions of community condition. Although biomass was positively associated with richness, its relationship with Shannon diversity was weaker, and biomass was additionally associated with community-weighted niche volume and culturability. Moreover, historical natural habitat availability was associated with richness but not biomass, despite the observed positive relationship between these two community properties. Together, these findings indicate that changes in the number and diversity of AM fungal taxa do not necessarily correspond to equivalent changes in the amount of fungal biomass maintained or the ecological strategies represented within communities. This distinction is important when considering AM fungi as indicators of soil health. Taxonomic richness does not necessarily predict ecosystem functioning because functional redundancy can weaken richness-function relationships, while community composition and functional traits may exert stronger effects on ecosystem processes (Bender et al., 2016; Delgado-Baquerizo et al., 2016). More generally, ecosystem processes depend not only on the number of taxa present but also on the amount and activity of biological biomass, and substantial functional variation can occur among AM fungal taxa within the same functional guild (Sikes et al., 2010; de Vries et al., 2013). DNA-based measures may also detect relic DNA and therefore do not necessarily distinguish active from inactive organisms (Fierer et al., 2021; Lekberg et al., 2022). Biomass measurements can therefore provide information on AM fungal community condition that is not captured by DNA-based diversity metrics alone.

Our findings consequently support a multidimensional approach to assessing AM fungal communities in soils. Combining taxonomic diversity with biomass and community ecological characteristics can distinguish changes in the number of taxa present, the amount of fungal biomass maintained, and the ecological strategies represented within communities. Importantly, these dimensions may respond differently to environmental drivers and retain signals of landscape change over different timescales. Integrating them should therefore provide a more comprehensive assessment of AM fungal responses to agricultural and landscape change, and potentially a more informative basis for their use as indicators of soil biological condition and ecosystem functioning.

5. Conclusions

Our findings demonstrate that AM fungal biomass provides information on community condition that is complementary to taxonomic diversity and community ecological characteristics. Relationships among these dimensions, and their contrasting responses to contemporary and historical landscape structure, indicate that no single metric fully captures AM fungal responses to environmental change. Integrating biomass, taxonomic diversity, and ecological characteristics, therefore, provides a more

comprehensive framework for assessing AM fungal communities and their potential contribution as indicators of soil biological condition in agricultural landscapes.

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