Cropping systems that improve richness convey increased resistance and resilience to fungal, but not prokaryote, communities.

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Keywords: Biodiversity; Community Dynamics; Conservation Agriculture; Arbuscular mycorrhizal fungi

Abstract

Resistance is the capacity for a community to remain unchanged, and resilience the capacity to return to an original state, in response to disturbance. Increasing species richness may increase both dynamics. In a long-term agricultural field experiment incorporating conventional (CON), integrated (INT), conservation (CA) and organic (ORG) cropping systems, the effects of crop harvest and fallow period on the disturbance of prokaryote, fungal and arbuscular mycorrhizal fungi (AMF) communities were investigated. It was hypothesised that: 1) change in composition observed over time due to disturbance differs between systems; 2) species-rich prokaryote communities demonstrate greater resistance and resilience than species-poor AMF; 3) key functional groups are more stable under systems that promote richness. Prokaryote community structure shifted over the growing season, forming distinct saprotroph- and rhizosphere-dominated communities over-winter and under mature crops, respectively. Management did not alter their response to disturbance, and they demonstrated the highest resistance/resilience. Fungal richness and resistance/resilience was highest under CA with a unique composition. AMF richness and resistance/resilience was lowest under CON with a fractured composition. Prokaryote plant-growth promoters and saprotrophs, but not ammonia oxidisers and methylotrophs, were stable functional groups. Diverse, cosmopolitan soil fungal genera were stable, but most were not. *Glomus* AMF were stable, while most other genera were stable under CA and ORG. These results demonstrate that practices promoting richness increase the stability of soil fungal communities, while prokaryote communities are more dynamic in structure. This may have consequences for the stability of fungal- and specific prokaryote-driven functions in response to crop harvest.

1. Introduction

Due to the global scale of anthropogenic impacts on natural ecosystems, the capacity for microbial communities to resist disturbance or return to an 'original' state is topical in microbial ecology. These concepts have been defined as: 1) resistance, which describes the capacity for a community to remain unchanged despite a disturbance event; and 2) resilience, which describes a dynamic process that considers the time it takes for a community to return to its original state after a disturbance event (Grimm and Wissel, 1997). Both of these concepts are entwined with the insurance hypothesis – communities that display greater species richness should also demonstrate greater resistance and resilience (Ives et al., 2000). Interestingly, despite the high diversity of microbial communities, most appear to be sensitive to disturbance and can take years or even decades to return to an original state (Allison and Martiny, 2008). In the spatially and temporally variable *in situ* environment, communities that display high richness, resilience and resistance may be more likely to ensure a stable rate of ecosystem processes.

The soil microbial community is primarily controlled by both plant-dependent factors and soil physico-chemistry (Philippot et al., 2013). It is a complex assembly including bacteria, archaea, obligate plant-symbiont arbuscular mycorrhizal fungi (AMF) and saprotrophic, non-mycorrhizal fungi. Bacteria regulate many essential processes, including nitrogen fixation (Rosswall, 1982), nitrification (Kowalchuk and Stephen, 2001), decomposition of complex organic matter (*i.e.* cellulose, hemicellulose, chitin) (Schimel and Schaeffer, 2012) and regulation of atmospheric greenhouse gases methane and nitrous oxide (Hanson and Hanson, 1996; Barnard et al., 2005). In the soil environment, Archaea produce methane and members of the phylum Thaumarchaeota oxidise ammonia (but with ammonia source) and pH often resulting

in niche differentiation from their bacterial counterparts (Prosser and Nicol, 2012). AMF are vital for growth of many plant species by facilitating nutrient (*e.g.* phosphorus and nitrogen) uptake from soil and transfer to roots (Koide and Kabir, 2000; Hodge and Fitter, 2010), but also improve soil aggregation and carbon sequestration via hyphal growth (Morris et al., 2019). Finally, saprotrophic fungi also assist in decomposition and recycling of complex organic matter (Setala and McLean, 2004). The combined activity of these microorganisms benefit plant-growth, often in exchange for root exudates as an organic carbon source (Cordovez et al., 2019).

In cropping systems, management practices have the potential to shape the composition of microbial communities and improve species richness. For example, tillage has a particularly detrimental effect on AMF community richness (Sale et al., 2015; Banerjee et al., 2019) and shift communities to favour Proteobacteria over Acidobacteria (Souza et al., 2013; de Vries et al., 2015). Mineral nitrogen fertilisation favours Actinobacteria, Proteobacteria and ammonia oxidising bacteria (AOB) over ammonia oxidising archaea (AOA) (Fierer et al., 2012). Organic fertilisation increases richness of Verrucomicrobia and Acidobacteria (Wessen et al., 2010). Pesticides have the potential to decrease nitrogen-fixing and nitrifying bacteria, among others (Johnsen et al., 2001). Permanent cover crops structure the community in many ways: a) their root systems provide stable soil moisture content that increases overall richness (Vukicevich et al., 2016); b) AMF-associated with cover crops remain stable in the soil, and interestingly can even transfer nutrients to commercial crops when they are present (Cheng and Baumgartner, 2006); and c) the rhizosphere of cover crops can promote root disease-suppressive Pseudomonads and fungal *Trichoderma* spp. (Vukicevich et al., 2016). A common

practice in perennial agricultural systems is cultivation of the crop followed by a fallow, intercrop period between sowing. Due to the importance of plant-microbe interactions in shaping the soil microbial community, the cultivation (*i.e.* removal) of an active root system likely acts as a dramatic disturbance event. Depending on the extent of community change (resistance) and the time it takes for the community to recover upon sowing for the next season (resilience) such a disturbance may have negative consequences for functional groups of microorganisms involved in various processes. Conversely, management practices that increase species richness may provide increased resistance and resilience to avoid detrimental effects of disturbance.

The La Cage long-term experimental agricultural site was established by the Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE) in 1998 in Versailles, France. Its purpose is to compare the performance of four cropping systems; conventional (CON) verses those with lower amounts of inputs - integrated (INT), conservation (CA) and organic (ORG). CA has been shown to increase soil organic carbon stocks, without increasing soil respiration rate, relative to other cropping systems (Autret et al., 2016). Additionally, CA improved bacterial, fungal, earthworm and arthropod biomass relative to CON, while only ORG increased bacterial biomass relative to other cropping systems (Henneron et al., 2015). The benefits of CA are thought to be primarily derived from the incorporation of a permanent cover crop, red fescue (*Festuca rubra*) followed by alfalfa (*Medicago sativa*). The La Cage site therefore represents a valuable model system to compare how various agricultural practices affect the resistance and resilience of soil microbial communities in response to crop cultivation. To address this, microbial community composition analyses were performed for prokaryotes, fungi and

specifically for AMF on CON, INT, CA and ORG soils over winter (December), half-way through the cropping season (May) and immediately prior to harvest (July). Specifically, it was hypothesised that: 1) the change in composition observed over time due to disturbance would differ between cropping systems; 2) species rich prokaryotes would demonstrate the greatest resistance and resilience, and species poor AMF would demonstrate the least, in response to crop harvest as a disturbance event; and 3) cropping systems that promote richness would provide increased resistance and resilience to key microbial functional groups in response to crop harvest as a disturbance event.

2. Materials and methods

2.1. La Cage experimental site

A detailed presentation of the La Cage long term trial has been reported previously (Debaeke et al., 2009). The site is located 15 km southwest of Paris (48◦48' N, 2◦08' E). The soil is a deep loamy, well-drained Luvisol (WRB, 2015). Texture includes: 15 – 18.4% clay (< 2 μm particle size diameter), 16.5 – 20.2% fine silt (2 – 20 μm), 30.3 – 43.2% coarse silt (20 – 50 μm) and 18.4 – 31.2% sand (50 – 200 μm). Table 1 outlines specific practices, crop production (wheat, pea and rapeseed t ha^{-1} yr^{-1}) and soil properties (total organic carbon TOC, total nitrogen TN, C:N ratio, pH and cation exchange capacity CEC) that differ between the cropping systems. Briefly, CON is typified by frequent tillage, pesticide application and high level of mineral N fertilisation. CA is no-till, infrequent pesticide application, has a permanent cover crop and receives N from mineral sources and a legume rotation. INT involves reduced tillage, infrequent pesticide application, and receives a reduced level of mineral N fertilisation. Finally, ORG involves frequent tillage, no pesticide application,

and receives N from a legume rotation. Wheat (*Triticum aestivum* L.) and rapeseed (*Brassica napus*) were grown on independent subplots (0.56 ha) under each cropping system across two blocks. For CON, INT and CA, a yearly rotating plot (12 m x 20 m) within each of the subplots did not receive a mineral N application. Rapeseed was sown in August and wheat in October, prior to any soil sampling, mineral fertiliser was applied in February, and crops were harvested in July. Wheat and rapeseed undergo annual rotations in these systems.

2.2. Soil sampling

Soil samples were collected in December 2015, May 2016 and July 2016 from the wheat and rapeseed subplots and no mineral N plots of the four cropping systems across both blocks. Five replicate soil samples were randomly taken in each subplot and plot, at a diameter and depth of 4 and 20 cm, respectively, using a hand auger, resulting in a total of 142 samples per collection date, and 426 samples overall. Soil samples were stored at -20°C.

2.3. Molecular analyses

DNA was extracted from 0.5 g soil using the MoBio PowerSoil DNA Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's protocol. Prokaryotic and AMF specific amplification of fragments of the Small Subunit rRNA gene (16S and 18S respectively) were carried out by using the primer pairs 515F/806R (Walters et al., 2015) and AMV4.5NF/AMDGR (Sato et al., 2005), respectively. Amplification of the fungal internal transcribed spacer 1 (ITS1) region was carried out with the primer pair ITS1F/ITS2 (Walters et al., 2015). Primers had Illumina adaptor sequences attached. PCR was performed in 27 µl reactions with 22.5 µl of Invitrogen Platinum PCR

SuperMix (Thermo Fisher, Carlsbad, CA, USA), 1.0 µl of forward and reverse primers (10 µM), and 2.5 µl of template DNA (5 ng/µl) on a Biometra T1 thermocycler (Biomentra GmbH, Göttingen, Germany). PCR conditions for 16S and ITS were as follows: 94°C for 3 min; 35 cycles at 94°C for 45 s, 50°C for 1 min, 72°C for 90 s; and 72°C for 10 min. For 18S, 95°C for 5 min; 35 cycles at 95°C for 45 s, 56°C for 45 s, 72°C for 1 min; and 72°C for 7 min was used. Amplicons were bead purified using Agencourt AMPure XP (Beckman Coulter, Villepinte, France), followed by indexing PCR using the Nextera XT Index Kit (Illumina, San Diego, CA, USA) following manufacture's recommendations. Indexed amplicons were bead purified using Agencourt AMPure XP and quantified using a µDrop Plate (Thermo Fisher, Carlsbad, CA, USA). Equimolar concentrations of samples were pooled and sequenced on an Illumina MiSeq sequencer with V2 2x150 bp paired-end chemistry. The raw sequences were deposited in the Sequence Read Archive; BioProject accession code PRJNA609408.

2.4. Bioinformatic analyses

Sequencing data was manually inspected using FastQC (Andrews, 2014), and processed using an established workflow, modified for fungal ITS and AMF 18S analyses. Sequence read pairs were first merged, using *vsearch* (Rognes et al., 2016) and thereafter trimmed to remove forward and reverse primers using *cutadapt* (Martin, 2015). Sequences lacking the correct primer sequences were discarded. Paired 16S sequences were then trimmed to a length of 252 and shorter sequences were discarded. For fungal ITS and AMF 18S sequences, with a more variable amplicon length, no trimming was carried out but sequences shorter than 100 or 200 bp, respectively, were discarded. Further, for all amplicons, sequences with more

than one expected error were discarded, using *vsearch.* Clustering and denoising was performed using Swarm v2 with default parameters (Mahé et al., 2015), followed by reference based and *de novo* chimera checking using *vsearch* (UCHIME algorithm; Edgar, 2012), with the RDP Gold reference sequences for 16S, SilvaMod v106 for 18S (Lanzén et al. 2012) and UNITE (Köljalg et al., 2005) for ITS. SWARM OTUs were then further subjected to clustering using *vsearch* with a cut-off of 97% minimum similarity, and singletons retained. Taxonomic classification of representative OTU sequences was then carried out using CREST (Lanzén et al., 2012) with the Silva v123 reference database (Quast et al., 2013), except for ITS for which the UNITE database was used (Köljalg et al., 2005). OTUs not identified as bacteria or archaea, fungi and Glomeromycota were removed from the Prokaryote 16S, fungal ITS and AMF 18S datasets, respectively. The MaarjAM database was used for AMF taxa identification of the OTUs to genera (similarity ≥ 97%, query coverage ≥ 90 %, E-value ≤ $1e^{-100}$) (Öpik et al., 2010).

2.5. Statistical analyses

All statistics were performed in R v3.5.2 (R Core Team, 2013). Initially Tukey's Honest Significant Difference (HSD) test was performed to test for differences in soil TOC, TN, C:N ratio, pH and CEC across cropping systems (n = 4 each).

Heatmaps showing the relative abundance (%) of Phyla within prokaryotes, fungi and AMF across time and cropping system were generated with the package 'gplots' (Warnes et al., 2019). Due to their extensive taxonomic diversity, Proteobacteria were displayed at the Class level. Analysis of variance was used to compare effects of time and management on the relative abundance (%) of each Phylum or Class.

Benjamin-Hochberg adjustments were made to *p* values to account for false discovery rate (Benjamini and Hochberg, 1995).

Samples with less than 4000 prokaryote or fungal sequences, and less than 400 AMF sequences, were removed from the datasets prior to the following analyses (2, 21 and 89 samples were removed, respectively). Rarefied species richness and Shannon indices were visualised with box and whisker plots across time and between management. Analysis of variance was performed to test for changes in richness and Shannon index across time and between management. Principle components analysis (PCoA) was performed on Bray-Curtis transformed dissimilarity OTU matrices, and permutational multivariate analysis of variance (perMANOVA) performed on Bray-Curtis transformed matrices with the *adonis* function, with 999 permutations, via the 'vegan' package (Oksanen et al., 2013).

For network analyses, prokaryote, fungi and AMF OTU matrices were subsetted based on individual management practice to compare OTU changes over time. Weighted networks were visualised from Spearman covariance matrices, with non-significant edge weights below the 95th quantile trimmed, performed with the 'igraph' package (Csardi and Nepusz, 2006). Nodes that lacked at least one edge were filtered out. Weighted networks were visualised with the force-directed Fruchterman-Reingold algorithm. Total nodes, edges, betweenness scores, clustering coefficients and diameter of each network was calculated as described (Csardi and Nepusz, 2006).

Jaccard Similarity was calculated for each sample over time, as a measure of resistance (July – December) and resilience (July – May). Jaccard Similarity (JS) was calculated as:

$$JS = \frac{\sum s_{ij}}{t_i + t_j} \qquad \text{Eq. 1}$$

where *s* was the sum of shared OTUs in samples *i* and *j*, while t_i and t_j were the total OTUs in samples *i* and *j*, respectively. JS was calculated at the genus level for prokaryotes and fungi, and at the individual OTU level for AMF due to their relatively low richness. The July (crop cultivation) time point for each sample was considered *i*, while *j* iterated through the December (over winter) and May (half-way through cropping) time points. The JS of wheat *versus* rapeseed crop were not compared, as each sample was only compared to its corresponding management/crop sample over time. Also of note is that JS compares similarity of species detection unweighted by relative abundance. A Kruskal-Wallis test was performed to confirm whether management practice affected JS over time. Finally, the proportional similarity of each prokaryote, fungal and AMF genus was calculated via Levins' niche breadth (B_N) (Levins, 1968). Here we consider each time point during community succession to be a separate niche. Specifically, B_N was calculated as:

$$B_N = \frac{1}{R} \sum_{i=1} p_i^2 \qquad \text{Eq. 2}$$

where *R* was the number of independent time points (3), and *p* was the proportional abundance of each genus in the i^{th} sample. A value of 1 indicates equal distribution of the taxon across time, whereas 1/*R* indicates the taxon is present at only one time point. For significance testing of proportional similarity values, null model testing of 999 randomly permuted OTU distributions was performed. The B_N of each genus was compared to this null distribution to derive a *p* value, which was Benjamin-Hochberg adjusted to account for false discovery rate (Benjamini and Hochberg, 1995). Further information regarding the use of B_N to test for proportional similarity can be found at 'github.com/DamienFinn/MicroNiche_Vignette', and the functions are available through the Comprehensive R Archive Network (CRAN) as the R package 'MicroNiche'.

3. Results

3.1. Soil properties and microbial community composition

Table 1 lists soil properties that differed between the four cropping systems. TOC was greatest under CA and lowest under ORG. Only CA had significantly greater TN than the other schemes. The C:N ratio, soil pH and CEC did not differ based on management.

Time was considered as a categorical variable with three levels: December sampling during winter with sown crops, May sampling half-way through the cropping season and July sampling just prior to crop harvest. Time primarily affected prokaryote phyla and proteobacterial classes, although management also affected several relatively abundant phyla including the Acidobacteria and Verrucomicrobia (Figure 1). Time was also the predominant effector of changes in fungal phyla abundance, with management affecting Basidiomycota and Glomeromycota. Time and cropping system affected AMF orders differently – time was important for the more dominant Glomerales and Diversisporales, whereas cropping system was important for the lesser abundant Archaeosporales and Paraglomerales. Prokaryote richness (1400 – 1600 OTUs) and Shannon (6.2 – 6.8) indices followed the trend Dec < May < July regardless of cropping system (Figure 2a). Time affected richness ($p < 0.001$) and Shannon index ($p = 0.01$), while cropping system affected neither ($p > 0.05$). Fungi richness (200 – 250 OTUs) was affected by time and cropping system ($p < 0.001$) with July and the CA system tending toward higher richness. This was reflected in the Shannon index (3 – 4) for time ($p < 0.001$) and cropping system ($p = 0.006$) (Figure 2b). The AMF richness (5 – 30 OTUs) and Shannon (0 – 3) differed within cropping system by CON < INT < CA < ORG and across time as Dec < May < July

(Figure 2c). All time and cropping system effects on AMF diversity indices were significant ($p < 0.001$). Figure 3 displays the PCoA of prokaryote (a), fungal (b) and AMF (c) community compositions. Prokaryotes were primarily affected by time (perMANOVA $R^2 = 0.22$, $p < 0.001$) and secondarily by cropping system (perMANOVA $R^2 = 0.07$, $p < 0.001$). This was evident by a clear separation of December communities (circles) versus May and July communities (triangles and squares). Fungi were affected foremost by cropping system (perMANOVA $R^2 = 0.15$, $p < 0.001$) than by time (perMANOVA $R^2 = 0.08$, $p < 0.001$). This was evident by the CA communities (blue) separating from all other communities under the other three practices. AMF were also affected by cropping system (perMANOVA $R^2 = 0.11$, $p < 0.001$) more than time (perMANOVA $R^2 = 0.05$, $p < 0.001$). This was evident by the CON communities (black) separating from all other communities under the other three cropping systems. Wheat and rapeseed crops had different communities but contributed less variance than time and cropping system (perMANOVA $R^2 = 0.01 – 0.03$, $p < 0.001$ for all groups). The plots without mineral N (perMANOVA $R^2 = 0.005 – 0.007$, $p < 0.002$ for all groups) and the sampling blocks (perMANOVA $R^2 = 0.002 – 0.004$, $p < 0.002$ for all groups) contributed the least to community differences.

3.2. Network analyses

Weighted networks of prokaryote, fungal and AMF communities were performed to support comparisons of relative abundances, diversity indices and ordination. Each node represents an OTU and each edge represents significant covariance between nodes based on Spearman covariance above the 95^{th} quantile of edge weights. Separate networks were constructed for each cropping system and include the three sampling times per network to visualise the effect of time on community covariance.

Table 2 lists node and edge numbers, mean and standard deviation of edge betweenness scores, clustering coefficients and the diameter of each network. Figure 4a are prokaryote networks for CON, CA, INT and ORG, respectively. Nodes and edges varied between cropping systems with lowest in ORG and highest in INT. Edge betweenness was also lowest in ORG and highest in INT. The clustering coefficients were high under all cropping systems (0.64 – 0.71) due to the formation of two distinct communities over time. Notably, one cluster tended to be dominated by Actinobacteria and Alphaproteobacteria, whilst the opposite cluster tended to host a diversity of Bacteroidetes, Gammaproteobacteria and other phyla. Figure 4b are fungal networks for CON, CA, INT and ORG, respectively. Nodes and edges were lowest in ORG and highest in CON. Edge betweenness was lowest in INT and highest in CON. Clustering coefficients were all low (0.39 – 0.46) due to a central core of fungal OTUs that did not vary over time surrounded by peripheral OTUs that did vary. Ascomycota and Basidiomycota dominated all fungal networks, indicating that only few Zygomycota OTUs covaried strongly with other OTUs. Figure 4c are AMF networks for CON, CA, INT and ORG, respectively. The least species rich AMF gave the sparsest networks with particularly low nodes in ORG and CON relative to CA. Similarly, ORG and CON had the lowest edges while CA was the most connected and had the highest edge betweenness values. The diameter of CON was high despite its low node number, indicating particularly sparse connectivity between nodes. Finally, the clustering coefficient of CON was also relatively high at 0.588, further suggestive of a less stable network over time compared to the low clustering coefficient of CA at 0.404.

3.3. Resistance and resilience of communities and functional groups over time

Figure 5a, b and c show JS indices for prokaryote, fungal and AMF communities over time. Resistance was considered as the JS between July and December, while resilience was considered as the JS between July and May. For prokaryotes, resistance remained consistent between cropping systems from the lowest ORG (0.47 – 0.6) to the highest CA (0.51 – 0.64). Resilience was also consistent from the lowest ORG (0.5 – 0.64) to the highest CA (0.51 – 0.67). There were no differences between cropping systems (Kruskal-Wallis p = 0.28). Resistance for fungi was lowest in CON (0.3 – 0.5) and highest in CA (0.45 – 0.56). Resilience for fungi was lowest in ORG (0.4 – 0.53) and highest in CA (0.45 – 0.58). Management had a slight effect on fungal resistance and resilience over time (Kruskal-Wallis p = 0.046). Finally, AMF resistance was lowest in CON (0 – 0.29) and highest in INT (0 – 0.41). Resilience was lowest in CON (0 – 0.51) and highest in CA (0.1 – 0.55). Cropping system affected AMF resistance and resilience over time (Kruskal-Wallis p = 0.028), with INT, CA and ORG practices outperforming CON.

3.4. Proportional similarity changes of functional groups

Table 3 lists B_N values for select genera involved in nitrogen fixation, plant-growth promotion, ammonia oxidation, methylotrophy and saprotrophy. A significantly low B_N indicates a genus that has a highly dissimilar distribution across time, which suggests its niche allows for its abundance to be high at only a specific time point during community succession. A significantly high B_N approaching 1 indicates a genus that is equally distributed across time points, which suggests its niche allows for generally equal abundance during community succession. Nitrogen fixers and plant-growth promoters were largely stable excepting for *Azospirillum* and *Rhizobacter*. These genera were present at multiple time points under CON and

were equally abundant. Abundances of AOA were affected by time. *Candidatus* Nitrosoarchaeum was only stable in CA and ORG. *Candidatus* Nitrosotalea was only present in CA. *Candidatus* Nitrososphaera was stable under most practices except ORG. The AOB *Nitrosospira* was transient under all practices, and absent from CA. The abundances of the AOB *Nitrosomonas* and *Nitrospira* persisted over time under all cropping systems. For methylotrophs, only methanotrophic *Methylobacter* and methylotrophic *Methylobacterium* were stable. The methanotroph *Methylocaldum* was only present under CA, and *Methylomicrobium* was stable except under ORG. Methylotrophic *Methylobacillus*, *Methylorosula* and *Methylotenera* were all transient under all cropping systems. For saprotrophs, *Actinomyces* was transient under all practices, and *Chitinivorax* was only present under CA. In general, however, the majority of prokaryote saprotrophs persisted stably through time.

Table 4 lists B_N values for saprotrophic and pathogenic fungal members of Ascomycota, Basidiomycota and Zygomycota. The fungi demonstrated less stability than the prokaryotes. Only four Ascomycota (*Mycosphaerella*, *Helotiaceae* spp., *Ophiosphaerella*, *Dendryphion*), one Basidiomycota (*Cryptococcus*) and two Zygomycota (*Coemansia*, *Ramicandelaber*) were unaffected by time or cropping system. Finally, Table 5 lists B_N values for AMF genera. Populations of *Glomus* remained stable across time under all cropping systems. *Claroideoglomus* and *Scutellospora* were stable under INT, CA and ORG. *Diversispora* was stable under INT and ORG. *Paraglomus* was stable under CON and INT. *Gigaspora* were only present in CA and ORG and differed markedly over time.

4. Discussion

4.1. General trends in high taxonomic ranks across time and cropping system

The removal of a crop during perennial harvesting is likely to act as a disturbance event for soil and rhizosphere communities in agricultural systems. This may result in significant disturbance to microbial groups that perform important functions in soils. It was hypothesised that: 1) management practice would affect how microbial community composition changed over time, as December (over-wintering with sown crop), May (mid-cropping season) and July (prior to harvest) in response to crop harvest as a disturbance event; 2) species rich prokaryotes would demonstrate greater resistance and resilience than species poor AMF in response to the removal of the crop; and 3) cropping systems that promote species richness would increase resistance and resilience of microbial communities in response to the removal of the crop.

In general, the dominant prokaryotic phyla in these soils reflected what is known of abundant phyla in agricultural systems. Bacteroidetes and Proteobacteria are dominant phyla in the wheat and rapeseed rhizosphere (Ai et al., 2015; Gkarmiri et al., 2017). Unsurprisingly, Bacteroidetes, Gammaproteobacteria and Betaproteobacteria decreased markedly in December in the absence of a mature plant host. These phyla tend to include copiotrophic taxa that are enriched by amino acids and monosaccharides (Fierer et al., 2007; Goldfarb et al., 2011; Ho et al., 2017) which are common root exudates that tend to be less available in bulk soils (Dennis et al., 2010; Kuzyakov and Blagodatskaya, 2015). Conversely, the Actinobacteria thrived in December (Figure 1). While this phylum is also present in both the wheat and rapeseed rhizosphere (Ai et al., 2015; Gkarmiri et al., 2017) it includes many saprotrophs that excel at degrading and consuming dead microbial and plant biomass (Barka et al., 2015). Thus, while the removal of the mature plant host proved detrimental to phyla that include many plant-microbe symbionts, it was

of benefit to saprotrophs. The Acidobacteria and Verrucomicrobia were the only dominant phyla affected by both time and management (Figure 1). Here they were enriched under ORG and INT practices. Both phyla are notably enriched under organic practices (Wessen et al., 2010; Ding et al., 2016; Li et al., 2017) while simultaneously decreasing under practices that involve adding mineral nitrogen (Fierer et al., 2012; Ramirez et al., 2012). Similar to the copiotrophic rhizosphere phyla, the abundance of Acidobacteria decreased in December. The Acidobacteria are also present in the wheat and rapeseed rhizosphere but may not necessarily grow directly on plant root exudates, as shown through isotope tracing (Ai et al., 2015; Gkarmiri et al., 2017). It was hypothesised that the Acidobacteria are specialised to utilise recalcitrant plant organic matter in the rhizosphere, such as sloughed root cells. Taken together, almost all dominant prokaryote phyla here were affected by the removal of the plant, with rhizosphere phyla decreasing in the absence of a mature crop and potential saprotrophs increasing under these circumstances.

The most abundant fungal phylum was the Ascomycota (Figure 1). This phylum frequently dominates cropping and pasture systems (de Castro et al., 2008; Klaubauf et al., 2010; Ma et al., 2013). Unlike the prokaryote phyla affected by time, here the abundance of Ascomycota decreased in July, prior to harvest, when the community was expected to be at its climax stage. The Ascomycota are a highly diverse phylum (Lutzoni et al., 2004). It includes saprotrophs (Voriskova and Baldrian, 2013), symbiotic endophytes of wheat and rapeseed (Abdellatif et al., 2009; Behie and Bidochka, 2014; Gkarmiri et al., 2017), and a number of pathogens of wheat and other crops, including the genera *Phaesophaeria*, *Alternaria* and *Fusarium* (Berbee, 2001). Fungal succession patterns during decomposition demonstrate preferential

enrichment of the Ascomycota at the beginning of plant degradation, which decrease over time (Voriskova and Baldrian, 2013; Vivelo and Bhatnagar, 2019). Presumably, endophytes and pathogens would both increase in abundance over time, and so it is tempting to speculate that the decrease of Ascomycota observed in July is reflective of saprotrophs being limited by the availability of specific plant substrates present in December and May. The second most abundant fungal phyla here were the Zygomycota. This differs from other agricultural systems that show Basidiomycota as the second most dominant group (de Castro et al., 2008; Klaubauf et al., 2010). The Zygomycota have been noted as dominant saprotrophs when plant organic matter is depleted of lignin relative to cellulose, toward the latter stages of decomposition (Osono and Takeda, 2001; Vivelo and Bhatnagar, 2019). Here, the effect of time on Zygomycota was inversely proportional to Ascomycota – that is, when Ascomycota decreased in relative abundance in July, the Zygomycota increased (Figure 1). Thus, fungal saprotroph succession may have primarily involved Ascomycota, followed by Zygomycota in these soils.

Finally, a more in-depth look at the AMF Glomeromycota identified Glomerales and Diversisporales to be the dominant orders. The overwhelming dominance of Glomerales in agricultural soils has been noted previously where three geographically separate soils under three different crops were dominated by > 90% Glomerales (Helgason et al., 1998). Woodlands were found to have much more even communities that included members of the Archaeosporales, Gigasporales and Diversisporales (Helgason et al., 1998). In arid agricultural soils the community can reflect roughly 50% Glomerales / 50% Diversisporales (Li et al., 2007) and abiotic stress (as fungicides, pollutants) also drive communities to be dominated by Glomerales and Diversisporales (Lenoir et al., 2016). However, it should be noted

that certain studies have found Glomerales and Paraglomerales to dominate agricultural systems (Sale et al., 2015; Banerjee et al., 2019). Time affected the Glomerales here, with greater abundances in July than December and May. This suggests Glomerales are the dominant obligate plant symbiotic AMF at the climax community just prior to harvest. Interestingly, brief 'blooms' of Diversisporales were apparent in December CON, CA, ORG and May CON, INT that coincided with a relative decrease in Glomerales abundance (Figure 1). A community shift to favour Diversisporales in December CA may be due to a symbiotic relationship between the permanent alfalfa cover crop. Alternatively, these small shifts may be a technical artefact arising from slight variation being more obvious within the low species rich (~ 250 OTUs) AMF community.

4.2. Time and cropping system effects on diversity and community composition

Firstly, it was hypothesised that cropping system would affect how communities changed through time in response to crop harvest. Only time, and not cropping system, affected prokaryote richness and Shannon biodiversity, with low biodiversity in December during the absence of a mature plant host and high biodiversity in July near the culmination of harvesting (Figure 2a). Although this study did not separately sequence the rhizosphere and bulk soil microenvironments, the presence of a mature plant clearly increased the biodiversity, which further increased from May to July prior to harvest. Time was also the greatest determiner of prokaryote community composition, with December communities distinct from May and July (Figure 3a). Interestingly, the prokaryote network analyses show the formation of two distinct clusters of significantly co-varying prokaryote taxa (Figure 4a) with high clustering coefficients (0.64 – 0.71). Regardless of cropping system, one cluster tended toward

low biodiversity dominated by potentially saprotrophic Actinobacteria and Alphaproteobacteria (the December cluster), while the other cluster tended toward higher biodiversity with all Proteobacteria classes, Bacteroidetes and Acidobacteria (the rhizosphere July and May cluster). Taken together, these three analyses indicate that while the absence of a mature plant decreased the overall biodiversity, the prokaryote communities were flexible in that a novel community was established. Then, in the presence of a mature plant, a distinct rhizosphere community emerged that increased in biodiversity over time until harvest in July. It is unclear whether prokaryote biodiversity is generally greater in the rhizosphere than bulk soil (Berg and Smalla, 2009; Philippot et al., 2013) with some studies suggesting selection for specific rhizosphere taxa reduces overall biodiversity (Ai et al., 2015) and others showing enriched biodiversity relative to bulk soil (McPherson et al., 2018). Here, the presence of a mature crop was clearly beneficial to prokaryote biodiversity and in shaping the community composition. Of note, however, is that the presence of the alfalfa cover crop in the CA system was not sufficient to maintain the biodiversity, community composition or network clustering in the absence of a mature commercial crop.

Fungi, in general, did not have the same time-dependent relationship as the prokaryotes. Biodiversity was high in both December and July (Figure 2b), and cropping system played a greater role in community composition (Figure 3b). Unlike the prokaryotes, the fungal community did not form distinct clusters of significantly covarying taxa across time – rather a central core of taxa was unchanged while other taxa at the periphery varied through time (Figure 4b). This central core was dominated by Ascomycota and Basidiomycota. Despite being the second most dominant phylum, only few individual Zygomycota nodes were in the networks,

scattered across the central core and periphery, indicating that only few Zygomycota covaried significantly with other taxa across time. This may be a consequence of the successional dynamics affecting the strength of taxon covariance across time. In terms of cropping system, the CA system had the most distinct community, which has been noted in fungal communities under no-till arable soils (Banerjee et al., 2019). Interestingly the ORG practice consistently had the lowest fungal biodiversity regardless of time, and demonstrated the lowest nodes (taxa), and edges (significant covariance) in community networks. The ORG practice had significantly lower TOC than the other practices and produced the lowest crop biomass t ha^{-1} $year^{-1}$. Conversely, the TOC-rich CA practice tended toward the highest fungal biodiversity. As soil TOC is predominantly plant C (Dungait et al., 2012) and assuming the majority of the Ascomycota, Basidiomycota and Zygomycota are saprotrophs reliant on plant debris from crops, the low TOC may be responsible for the decreased biodiversity and network complexity observed in the ORG practice. At the global scale, climate and plant diversity are the most important drivers of fungal biodiversity (Tedersoo et al., 2014). However, at more local scales, for example within northern China and within the Mediterranean, TOC is the most important driver of fungal biodiversity (Persiani et al., 2008; Liu et al., 2015). The abundance of Ascomycota and Zygomycota phyla were also shown to be particularly dependent on TOC (Liu et al., 2015). Thus, crop productivity, TOC and no-till likely influenced overall fungal biodiversity, composition and network properties.

The biodiversity of AMF followed clear trends of increasing from December, through May to July prior to harvest, with the CON system demonstrating consistently poor richness and Shannon indices. Furthermore, community composition under the CON practice was dissimilar to the other practices and the structure of the CON network

sparser with low edge betweenness and high diameter than the other cropping systems. The CON and ORG system, where tillage occurred annually, both had the lowest nodes, edges and edge betweenness scores of the AMF networks. Tillage, and frequent pesticide application in the case of CON, have the potential to severely limit the biodiversity of AMF (Sale et al., 2015; Gottshall et al., 2017; Banerjee et al., 2019). Recently, Banerjee et al. (2019) found a negative relationship between increasingly intensive agricultural management practices and node, edge and edge betweenness values within AMF community networks. Here the CA network had the greatest nodes, edges and edge betweenness values and corresponded to the least intense practice as no-till, whilst also incorporating an alfalfa cover crop that could further benefit AMF biodiversity (Vukicevich et al., 2016). Therefore, the biodiversity, community composition and network properties of the AMF were in strong agreement with previous research on positive and negative drivers of AMF communities.

4.3. Resistance and resilience of prokaryotes, fungi and AMF

The factors that confer resistance and resilience to soil microbial communities have received a great deal of attention due to the role of microbes as drivers of ecosystem processes (Griffiths and Philippot, 2012; and references therein). Disturbance can come in many forms – drought (Manzoni et al., 2012), fire (Banning and Murphy, 2008), metal (Philippot et al., 2008) and organic pollutants (Girvan et al., 2005). Increasing biodiversity of a community can strengthen its capacity for resistance and resilience in response to a disturbance event, termed the ‘insurance hypothesis’ (Ives et al., 2000). Here we considered crop harvesting followed by a fallow period to be a disturbance, with four cropping systems that may promote resistance and

resilience of microbial communities. As hypothesised, the species rich prokaryotes demonstrated the highest resistance (56% similarity to July) and resilience (60% similarity to July) in response to crop removal over summer-autumn. Fungi were less resistant (45%) and resilient (49.6%), with soils under CA showing slightly better capacity for resilience than the average (51.3%, $p = 0.046$). Finally, as hypothesised, the species poor AMF demonstrated the lowest resistance (20%) and resilience (31.8%), with soils under CON having a particularly poor capacity for resilience than the average (23.6%, $p = 0.028$). Thus, the resistance and resilience of prokaryotes, fungi and AMF broadly agreed with the insurance hypothesis. In addition to the richness of a community, several other factors are important contributors to resistance and resilience. For example, sampling time during rhizosphere community development is particularly important, and it can take over 16 months for a disturbed community to return to its undisturbed state (Orwin and Wardle, 2005). This is a likely contributor to why the prokaryotes only reached 60% similarity to end of harvest when sampled mid-way through the cropping season in May. Furthermore, soil aggregation and TOC can provide isolated microhabitats and/or support growth of micro-organisms post-disturbance, and these edaphic properties have been strongly linked to resilience (Griffiths et al., 2005; Zhang et al., 2010). As soils under CA were no-till, and likely had better soil aggregation than annually tilled CON and ORG (West and Post, 2002), this may explain the higher fungal resistance and resilience observed. Similarly, annual tillage and frequent pesticide application may have significantly reduced the resistance and resilience of AMF communities under CON. Finally, it is possible that the resistance and resilience of all communities measured here are high relative to other forms of disturbance because of adaptation after previous crop harvesting and fallow period (Griffiths and Philippot, 2013). With

the exception of the cover crop in CA soils, each practice endures an annual fallow period which has been ongoing for approximately 20 years. This may act as a form of pressure to strictly select for microbial species that can survive during this fallow period in the absence of an active rhizosphere. Even so, crop removal had dramatic effects on the composition of all communities, particularly the AMF.

4.4. Cropping system effects on stability of key microbial functional groups

Finally, we hypothesised that certain microbial functional groups would demonstrate less resistance and resilience than others. This was measured with Levins' proportional similarity index (B_N), where a value approaching 1 indicated the abundances of a taxon were 'stable' across the three time points, a value approaching 0.333 indicated a taxon was unique to a single time point, and values between indicated an uneven distribution across time. Where B_N was significantly low, we interpreted this as a taxon with an 'unstable' distribution across time.

Functions can be broadly classified as 'broad' or 'narrow' (Schimel and Schaeffer, 2012). Broad functions are carried out by a relatively large proportion of the community, such as decomposition, whereas narrow functions are carried out by a small subset of the community as part of an obligate metabolic pathway, such as ammonia oxidation or methylotrophy (Graham et al., 2016). In the prokaryotes, stable community members whose abundances persisted over time, regardless of cropping system, included numerous nitrogen-fixers, plant-growth promoters and saprotrophs, with a notable exception of *Actinomyces* of the Actinobacteria, that was only detectable in December. Populations of ammonia oxidisers and methylotrophs differed markedly over time, indicating that narrow functions could be more affected by crop harvest. Furthermore, the CA soils uniquely supported stable populations of

the AOA *Ca*. Nitrosoarchaeum and *Ca*. Nitrososphaera (Schleper and Nicol, 2010), whilst also selecting against *Nitrosospira* (Lourenco et al., 2018). The CA soils also supported the most rich, stable populations of methanotrophs *Methylobacter*, *Methylocaldum* and *Methylomicrobium* (Ho et al., 2013). Therefore, while cropping system did not affect overall community resistance and resilience in prokaryotes, a closer look at the stability of ammonia oxidisers and methylotrophs indicated that the CA practice was most beneficial to these narrow functional groups.

The fungi in Table 4 are a subset of saprotrophs (Voriskova and Baldrian, 2013) and potential wheat pathogens, such as *Alternaria*, *Phaeosphaeria*, *Mycosphaerella* and *Septoria* (Shipton et al., 1971; Berbee, 2001). Although saprotrophs can be considered a broad functional group, populations of most fungal genera shifted markedly over time under one or more cropping systems. Those that remained stable, such as *Mycosphaerella*, *Helotiaceae* spp. and *Cryptococcus*, are highly diverse genera that are cosmopolitan across diverse soils (Vishniac, 1995; Crous et al., 2007; Zheng and Zhuang, 2015). Others, such as *Coemansia*, are typical of the wheat rhizosphere (Kwasna et al., 1999). Genera that were specific to certain cropping systems and/or affected by time may have been: a) saprotrophs involved in the Ascomycota-to-Zygomycota succession; b) genera involved in specialist functions such as *Actinomucor* producers of unique aminopeptidases (Ito et al., 2003); or c) pathogens such as *Septoria* and *Phaeospaeria* that were absent under systems that promoted high biodiversity, which can result in disease suppression (Penton et al., 2014).

Lastly, Table 5 lists how time and cropping system affected each AMF genus. The only stable genus across time and system was *Glomus*, a large, diverse and polyphyletic group of AMF (Schwarzott et al., 2001). *Glomus* includes multiple

endophytic species of wheat (Al-Karaki et al., 2004) involved in the hydrolysis and transfer of organic and inorganic phosphorus to host plants (Koide and Kabir, 2000), and so the persistence of this genus across time, regardless of cropping system, is likely to be valuable to the crop. While rapeseed is known for not associating with AMF, specific species such as *Glomus caledonium* can infect rapeseed roots (but with poor germination, appressorial formation and hyphal growth) (Tommerup, 1984) and so crop rotations between wheat and rapeseed over time may be another factor in selecting for *Glomus* spp. with broad host range. The stability of other AMF genera was linked to the cropping systems that demonstrated the greatest AMF resilience – *Claroideoglomus* and *Scutellospora* were stable under INT, CA and ORG, *Diversispora* were stable under INT and ORG, and *Gigaspora* was stable under CA and ORG. *Gigaspora* and *Scuttelospora* have been identified as specialist AMF genera that prefer less intensive agricultural practices (Sale et al., 2015; Gottshall et al., 2017; Banerjee et al., 2019) while *Paraglomus* spp. have been shown to be largely unaffected by tillage (Sale et al., 2015). The specific role that these less stable AMF taxa may play in promoting plant growth is unclear, however multiple AMF genera can infect the same host simultaneously, and indeed the composition of the endophytes can contribute to greater or lesser plant growth (Chen et al., 2017). Therefore, the INT, CA and ORG systems that promoted stable populations of diverse AMF genera may be beneficial relative to the CON system.

While specific measurements for decomposition, ammonia oxidation, methanotrophy, methylotrophy and AMF nutrient transfer were not measured in this study, the link between richness and a functionally redundant community that can provide a stable rate of an ecosystem process under varying environmental conditions has been described (Allison and Martiny, 2008). Increasing biodiversity is

associated with broad functions such as increasing plant productivity, nitrification and decomposition (Delgado-Baquerizo et al., 2016; Graham et al., 2016; Hazard and Johnson, 2018). In response to disturbance and species loss, diversity-poor communities are unable to maintain rates of cellulose hydrolysis, 2,4-dichlorophenol metabolism or fungal-driven decomposition (Setala and McLean, 2004; Wohl et al., 2004; Girvan et al., 2005). Conversely, methane oxidation, nitrite oxidation and denitrification rates have been shown to be maintained despite species loss (Wertz et al., 2007; Ho et al., 2011). The vast majority of experimental evidence to support linking biodiversity, disturbance and an ecosystem process have been conducted *in vitro*, and consequently may underrepresent the importance of richness in the spatially and temporally heterogeneous soil environment *in situ*. Therefore, the broader relevance of this study is that certain narrow prokaryote functions may be most stable under CA, fungal saprotrophy may be more stable under CA, and AMF plant symbioses most stable under CA, INT or ORG.

5. Conclusions

Presented here is a thorough investigation of prokaryote, fungal and AMF soil communities that demonstrated markedly different responses to crop harvest as a disturbance event under four agricultural cropping systems. Prokaryotes were flexible and formed a distinct saprotroph-dominated community in the absence of a mature crop, and a rhizosphere-dominated community in the presence of a mature crop. This relatively species rich group demonstrated the highest resistance and resilience, although narrow functional groups were affected by cropping system and were less stable over time. The CA system was associated with the most structurally diverse fungal community, which also increased resistance and resilience. While

some cosmopolitan soil genera remained stable, the majority of fungi were unstable between managements and across time. Finally, the CON practice had a detrimental effect on AMF richness, resistance and resilience. *Glomus* spp. were stable between cropping systems and across time, while CA and ORG systems promoted richness and stability of other genera. In conclusion, cropping systems that promoted fungal richness led to increased stability of general fungal and AMF communities, in addition to some narrow functional groups of prokaryotes.

Acknowledgements

We would like to thank INRAE, Versailles-Grignon for use of the La Cage field trial, and Gilles Grandeau for assistance with soil sampling. This project was funded by the AXA Research Fund.

Conflict of interest statement

The authors declare no conflict of interest in regard to the outcomes of this study.

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Table 1: Management practices, crop production and soil properties of the La Cage cropping systems. Superscript letters indicate Tukey's HSD outcomes between management schemes at the $p < 0.05$ level.

	CON	INT	CA	ORG
Tillage	Annual	Biannual	None	Annual
N fertilisation	Mineral-high	Mineral-reduced	Mineral-reduced	None
Pesticides	Frequent	When nec.	When nec.	None
Cover crop	No	No	Yes	No
Legume rotation	No	No	Yes	Yes
Wheat (t ha^{-1} yr^{-1})	9.7	8.9	6.7	5.4
Pea (t ha^{-1} yr^{-1})	4.2	4.5	3.7	2.6
Rapeseed (t ha^{-1} yr^{-1})	4.5	3.8	-	0.8
TOC	$11.1 \pm 0.9^{a,b,c,d}$	$11 \pm 1.6^{a,b,c,d}$	$13.2 \pm 2.8^{a,b,c}$	$10.4 \pm 0.5^{a,b,d}$
TN	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	1.1 ± 0.2^{b}	0.9 ± 0.1^{a}
C:N ratio	12.4 ± 1^{a}	11.8 ± 0.2^{a}	11.5 ± 0.1^{a}	11.6 ± 0.3^{a}
pH	7.3 ± 0.3^{a}	7.1 ± 0.4^{a}	6.9 ± 0.2^{a}	7 ± 0.2^{a}
CEC	9.9 ± 1^{a}	9.5 ± 1^{a}	9.6 ± 0.3^{a}	9.2 ± 0.3^{a}

Soil properties were measured in all subplots in 2014 at INRAE.

Table 2: Network properties separated by cropping system.

		CON	INT	CA	ORG
Prokaryotes	Nodes	440	464	432	391
	Edges	15603	16735	15260	11739
	Betweenness	290 ± 328	297 ± 301	282 ± 352	280 ± 353
	Clustering coef.	0.644	0.646	0.651	0.709
	Diameter	1431	1377	1313	1480
Fungi	Nodes	285	246	259	236
	Edges	6079	5167	4812	3923
	Betweenness	161 ± 152	133 ± 117	145 ± 135	139 ± 127
	Clustering coef.	0.439	0.458	0.396	0.453
	Diameter	469	429	485	184
AMF	Nodes	85	118	151	85
	Edges	721	1134	1512	663
	Betweenness	53 ± 63	77 ± 113	98 ± 122	54 ± 65
	Clustering coef.	0.588	0.474	0.404	0.501
	Diameter	351	362	296	157

Table 3: Proportional similarity (B_N) differences across time, within cropping system, for prokaryote genera involved in nitrogen fixation, plant-growth promotion, ammonia oxidation, methylotrophy and saprotrophy. Significance is marked as: $p < 0.05$ (*); $p = 0.001$ (**); $p < 0.001$ (***).

	CON		INT		CA		ORG	
Nitrogen fixers and plant-growth promoters	B_N	BH *p* val	B_N	BH *p* val	B_N	BH *p* val	B_N	BH *p* val
Azospirillum	0.857	0.651	-	-	0.533	0.011*	0.333	0.000***
Bacillus	0.929	0.266	0.905	0.359	0.927	0.259	0.867	0.580
Bradyrhizobium	0.826	0.855	0.860	0.620	0.943	0.210	0.956	0.165
Burkholderia	0.832	0.820	0.884	0.469	0.883	0.472	0.599	0.062
Mesorhizobium	0.908	0.356	0.866	0.579	0.882	0.479	0.794	0.943
Phyllobacterium	0.807	0.976	0.900	0.383	0.928	0.255	0.764	0.753
Pseudomonas	0.703	0.388	0.662	0.207	0.621	0.101	0.821	0.890
Rhizobacter	0.667	0.226	0.333	0.000***	-	-	0.333	0.000***
Rhizobium	0.802	0.996	0.775	0.808	0.897	0.402	0.902	0.381
Rhizocola	0.764	0.763	0.694	0.327	0.777	0.821	0.584	0.044*
Rhizomicrobium	0.845	0.737	0.903	0.366	0.977	0.119	0.939	0.212
Rhizorhapis	0.801	0.986	0.921	0.284	0.727	0.505	0.617	0.089
Ammonia oxidisers								
Ca. Nitrosoarchaeum	0.513	0.007**	0.490	0.003**	0.667	0.217	0.600	0.062
Ca. Nitrososphaera	0.617	0.100	0.589	0.055	0.670	0.230	0.583	0.043*
Ca. Nitrosotalea	-	-	-	-	0.333	0.000***	-	-
Nitrosomonas	0.718	0.471	0.687	0.297	0.788	0.896	0.630	0.114
Nitrosospira	0.333	0.000***	0.333	0.000***	-	-	0.333	0.000***
Nitrospira	0.877	0.530	0.910	0.332	0.991	0.090	0.998	0.073
Methylotrophs								
Methylobacillus	0.462	0.001**	0.434	0.000***	0.521	0.008**	0.333	0.000***
Methylobacter	0.656	0.195	0.651	0.172	0.990	0.092	0.667	0.212
Methylobacterium	0.799	0.979	0.812	0.934	0.695	0.337	0.614	0.083
Methylocaldum	0.333	0.000***	0.533	0.012*	0.600	0.067	-	-
Methylomicrobium	0.843	0.747	0.610	0.083	0.711	0.419	-	-
Methylorosula	-	-	0.333	0.000***	-	-	-	-
Methylotenera	0.600	0.070	0.333	0.000***	0.333	0.000***	0.333	0.000***
Saprotrophs								
Actinomyces	0.333	0.000***	0.333	0.000***	0.333	0.000***	0.333	0.000***
Bryobacter	0.914	0.328	0.957	0.164	0.990	0.092	0.997	0.074
Ca. Solibacter	0.977	0.121	0.999	0.078	0.997	0.080	0.988	0.089
Ca. Koribacter	0.935	0.241	0.994	0.084	0.959	0.161	0.972	0.121
Cellulomonas	0.864	0.611	0.712	0.422	0.714	0.433	0.664	0.212
Chitinibacter	0.475	0.002**	0.947	0.193	0.659	0.202	0.887	0.459
Chitinivorax	-	-	-	-	0.776	0.808	-	-
Chitinophaga	0.854	0.668	0.957	0.164	0.806	0.976	0.779	0.855
Polyangium	0.986	0.103	0.910	0.334	0.960	0.160	0.909	0.345
Sorangium	0.774	0.822	0.739	0.574	0.755	0.675	0.680	0.268

Streptomyces	0.770	0.803	0.783	0.862	0.858	0.626	0.623	0.099
Terribacillus	0.646	0.164	0.756	0.676	0.604	0.074	0.462	0.001**
Terrimicrobium	0.803	1.000	0.666	0.213	0.930	0.249	0.811	0.943
Terrimonas	0.909	0.354	0.936	0.224	0.943	0.209	0.941	0.212
Verrucomicrobium	0.973	0.130	0.678	0.261	0.753	0.660	0.746	0.632

Table 4: Proportional similarity (B_N) differences across time, within cropping system, for saprotrophic and pathogenic fungal genera. Significance is marked as: $p < 0.05$ (*); $p = 0.001$ (**); $p < 0.001$ (***).

	CON		INT		CA		ORG	
Fungi	B_N	BH *p* val	B_N	BH *p* val	B_N	BH *p* val	B_N	BH *p* val
Ascomycota								
Capnodiales								
Capnodium	0.619	0.082	0.333	0.000***	0.468	0.001**	0.600	0.052
Cladosporium	0.847	0.701	0.823	0.856	0.806	0.974	0.471	0.001**
Mycosphaerella	0.760	0.714	0.817	0.902	0.872	0.542	0.772	0.776
Ramularia	0.333	0.000***	0.393	0.000***	0.421	0.000***	0.333	0.000***
Septoria	-	-	-	-	-	-	0.333	0.000***
Sphaerulina	0.356	0.000***	0.638	0.135	0.600	0.056	-	-
Stigmina	0.662	0.188	0.333	0.000***	0.343	0.000***	0.333	0.000***
Helotiales								
Articulospora	0.564	0.021*	0.682	0.266	0.663	0.197	0.762	0.715
Helotiaceae spp.	0.638	0.123	0.680	0.255	0.724	0.484	0.953	0.159
Hymenoscyphus	0.563	0.021*	-	-	-	-	0.333	0.000***
Neobulgaria	0.857	0.632	0.832	0.802	0.571	0.027*	0.655	0.168
Pleosporales								
Corynespora	0.637	0.120	0.333	0.000***	0.926	0.254	0.505	0.003**
Leptosphaeria	0.741	0.593	0.601	0.060	0.629	0.102	0.478	0.001**
Lophiostoma	0.778	0.843	0.490	0.002**	0.690	0.296	-	-
Ophiosphaerella	0.929	0.236	0.976	0.114	0.793	0.940	0.950	0.168
Paraphaeosphaeria	0.600	0.054	0.333	0.000***	-	-	0.333	0.000***
Phaeosphaeria	0.342	0.000***	0.708	0.395	-	-	0.459	0.001**
Alternaria	0.376	0.000***	0.462	0.001**	0.407	0.000***	0.359	0.000***
Bipolaris	-	-	0.333	0.000***	-	-	0.855	0.640
Chalastospora	0.616	0.078	0.458	0.001**	0.633	0.112	0.333	0.000***
Curvularia	0.621	0.085	0.600	0.059	-	-	-	-
Dendryphion	0.958	0.147	0.771	0.790	0.969	0.118	0.888	0.437
Drechslera	0.333	0.000***	-	-	-	-	0.333	0.000***
Epicoccum	0.530	0.008**	0.472	0.001**	0.407	0.000***	0.563	0.019*
Pleospora	-	-	0.333	0.000***	0.333	0.000***	-	-
Basidiomycota								
Tremellales								
Sirobasidium	-	-	0.333	0.000***	0.333	0.000***	-	-

Bandoniozyma	0.543	0.011*	-	-	-	-	0.333	0.000***
Bullera	0.600	0.054	0.665	0.207	0.396	0.000***	0.664	0.196
Bulleromyces	0.365	0.000***	0.333	0.000***	0.621	0.088	0.533	0.008**
Cryptococcus	0.858	0.629	0.931	0.237	0.739	0.580	0.808	0.954
Dioszegia	0.808	0.965	0.387	0.000***	0.777	0.836	0.652	0.158
Hannaella	0.407	0.000***	0.563	0.023*	0.533	0.009**	0.333	0.000***
Zygomycota								
Mucorales								
Actinomucor	-	-	0.600	0.059	-	-	-	-
Mucor	0.707	0.379	0.508	0.004**	0.419	0.000***	0.517	0.005**
Rhizopus	0.333	0.000***	-	-	0.333	0.000***	0.333	0.000***
Kickxellales								
Coemansia	0.752	0.654	0.976	0.115	0.748	0.647	0.863	0.586
Linderina	0.333	0.000***	0.333	0.000***	-	-	0.333	0.000***
Ramicandelaber	0.635	0.117	0.712	0.411	0.652	0.162	0.722	0.468

Table 5: Proportional similarity (B_N) differences across time, within cropping system, for AMF genera. Significance is marked as: $p < 0.05$ (*); $p = 0.001$ (**); $p < 0.001$ (***).

	CON		INT		CA		ORG	
AMF	B_N	BH *p* val	B_N	BH *p* val	B_N	BH *p* val	B_N	BH *p* val
Archaeospora	0.964	0.124	0.956	0.283	0.585	0.048*	0.605	0.075
Claroideoglomus	0.375	0.000***	0.825	0.811	0.929	0.207	0.588	0.064
Diversispora	0.492	0.002**	0.587	0.093	0.517	0.008**	0.707	0.358
Gigaspora	-	-	-	-	0.512	0.008**	0.340	0.000***
Glomus	0.700	0.324	0.914	0.387	0.773	0.752	0.839	0.704
Pacispora	0.407	0.000***	0.752	0.679	0.618	0.066	0.693	0.358
Paraglomus	0.797	0.952	0.854	0.679	0.508	0.008**	0.451	0.001**
Scutellospora	0.480	0.002**	0.938	0.309	0.601	0.051	0.903	0.358
unclassified	-	-	0.333	0.000***	0.592	0.048*	-	-

Figure legends

Figure 1: Heatmap comparing relative abundances of prokaryote and fungal phyla, and AMF orders, across time and between cropping system. Where phyla differed over time (T) or by cropping system (C) ANOVA results are shown as: $p < 0.05$ (*); $p = 0.001$ (**); $p < 0.001$ (***).

Figure 2: Observed OTU richness and Shannon indices for: a) prokaryotes, b) fungi and c) AMF.

Figure 3: PCoA ordinations of community composition for: a) prokaryotes, b) fungi and c) AMF. Variance explained by axes 1 and 2 are noted on *x* and *y* axes, respectively.

Figure 4: Weighted networks of a) prokaryotes under CON, INT, CA and ORG; b) fungal networks under CON, INT, CA and ORG; and c) AMF networks under CON, INT, CA and ORG. Nodes of major taxonomic groups have been coloured and enlarged.

Figure 5: Plots of JS over time and between cropping system for: a) prokaryotes, b) fungi and c) AMF.