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Abstract

Background and Aims:

Agroecology practices can induce profound changes in soil inevitably influencing soil biological properties and soil functioning. However, we still lack understanding of how soil biodiversity responds to agroecology practices and to what extent such practices, alone or combined, can be beneficial for soil functioning. Understanding soil biological activities under different agroecology practices is important for predicting carbon cycling in agroecosystems.

Methods:

By taking advantage of a long-term agricultural experimental research station in France, we monitored soil microbes, nematodes and soil respiration over three years in response to agroecology practices that varied in the rate of nitrogen (N) fertilization (low vs high), the tillage type (deep vs reduced), and the crop residue management (retain vs removal).

Results:

Shifting from conventional to agroecology practices had strong effects on microbial biomass, nematode community and soil respiration. Reduced N and reduced tillage increased microbial biomass carbon, bacterivore and fungivore density. Perennial biomass crop decreased total nematode and herbivore density, but increased microbial biomass. Perennial biomass crop also significantly increased the structure and maturity indices, but decreased the plant parasite indices. Structural equation modelling showed that microbial biomass had a positive correlation with soil respiration in reduced nitrogen, reduced tillage, and residue removal treatments. Bacterivores had a positive correlation with omnivores/predators and soil respiration, while herbivores had a negative correlation with soil respiration in all the treatments.

Conclusions:

The different agroecological practices tested in this 4-year trial revealed the resilience of nematode communities and associated functions like CO2 respiration according to practices.

1. Introduction

Long-term conventional agricultural practices were shown to simplify landscapes, disturb soil structure and increase greenhouse gas emissions (Chabert & Sarthou, 2020; Akakpo et al., 2021). New challenges to global food security, a primary concern for humanity, are posed by global change and the urgent need to achieve this goal in a sustainable manner for the next centuries. Agroecological approaches that seek to promote agricultural production in order to meet the increasing demand of an exponentially growing human population, and ecosystem services for mitigating climate change, for example, are ways to favour sustainable agricultural productivity (Pittelkow et al., 2015). Agroecology is the use of environmentally friendly farming practices, which has been argued to sustain crop productivity, increase food security, and enhance soil nutrient availability (Coulibaly et al., 2017; Pittelkow et al., 2015; Akakpo et al., 2021). Diversifying crop rotation, agroforestry, cover cropping, and soil management measures (such as reducing tillage) are all agroecological practices (Kerr et al., 2021). These practices can induce profound changes in soil physical and chemical properties, and inevitably influence soil biological properties and soil fertility, as well as soil functioning, over time (Panettieri et al., 2020).

By contributing to about 10% of the global soil respiration (Yu et al., 2018), agricultural soils are important sources of carbon dioxide (CO_2) emissions. Previous studies identified management practices, such as N fertilization (Yan et al., 2021), tillage (Faust et al., 2019) and cover with crop residues (Buysse et al., 2013) as driving factors of such greenhouse gas emissions. However, we still lack understanding of how soil biodiversity responds to agroecological practices; to what extent such practices, alone or in combination, can be beneficial for soil functioning; and what services they can provide (Puissant et al., 2021). Understanding soil respiration and their biological determinants under different agroecology practices is important for predicting carbon cycling in agroecosystems.

Nematodes are one of the most abundant and diverse invertebrates in the soil. Their vast genetic diversity, phenotypic plasticity and feeding habits enable them to colonise and occupy a great number of habitats (van Megen et al., 2009, Viney & Diaz, 2012, Van den Hoogen et al., 2019). Nematodes are divided into different feeding groups covering trophic levels from bacterivores to predators and herbivores (Yeates et al., 1993). Furthermore, according to their life-history strategies, free-living nematodes are classified into 5 groups on a coloniser-persister continuum (Bongers, 1990). Group 1 is made of coloniser species corresponding to r-strategy species, while group 5 is composed of K-strategy species known as persisters (Bongers, 1990). The relative abundance of either feeding groups or life-history groups was successfully used to calculate different micro food web indices (e.g. the maturity indices, the enrichment indices) that provide a direct overview of soil functioning state (Ferris and Matute, 2003; Ferris et al., 2010). In a cropping context, free-living nematodes had a close link with bacteria and fungi in soil food webs, and their interactions had consequences for soil organic matter decomposition, and therefore for carbon and nutrient cycling (Jiang et al., 2017; Sun et al., 2019). Recently, Jiang et al. (2018) suggested that bacterivore grazing had a significant impact on the turnover rates of soil organic carbon (SOC) pools at the aggregate scale through the bacteria/fungi ratio.

As recently noted in a meta-analysis by Puissant et al. (2021), much work has been performed over the last 50 years on describing soil nematodes communities in croplands. Their results demonstrate that, while changing the tillage system generated a relative weak response, chemical inputs (both fertilizers and pesticides) had the strongest effects on soil nematodes at the global scale. High mineral fertilization rates induced lower richness and diversity of nematodes (Puissant et al., 2021). Crop straw is rich in organic material, and straw application could improve the aggregate structure and stability (Karami et al., 2012). Thus, crop residue practices could change the soil nematode community and structure. There is also a need to consider the temporal aspects of agricultural practices that have been adopted, because nematodes at the higher trophic level need a long period to be re-established after changes in

management strategies (Ferris, 2010, Villenave et al. 2018). However, the number of studies addressing the effects of conversion of practices (multi factor effects) and not only a single factor effect (e.g., tillage, pesticides, fertilization) is rather low – less than 5 to 10, according to the variable considered. This clearly indicates the importance of pursuing our effort to document how agricultural practices or management may affect soil nematode communities and their associated functions over time.

The overall objective of this study was to identify how agroecology practices modified the soil microbe and nematode communities, and how this affected soil respiration. By taking advantage of a long-term agricultural experimental research station located in northern France, we monitored, over a period of 3 years, soil microbes, nematodes and soil respiration in response to conversion of conventional agricultural practices towards different agroecology practices linking the rate of N fertilization (low vs high), the tillage type (deep vs reduced), and the crop residue management (retain vs removal). We hypothesised that (1) reduced N fertilization and reduced tillage would increase both maturity and structure indices (i.e. indicating a more complex soil food web) as well as increase microbial enzymatic activities, as both fertilization and tillage are known to disturb the soil system favouring opportunistic or r-species; (2) crop residue removal would negatively impact soil nematode community, by reducing the alpha diversity and the Enrichment indices, for example; (3) perennial crops would increase nematode density, maturity indices and the structure indices by strongly reducing external soil disturbances and prompting successional processes that favour K-strategist species, with this becoming more and more obvious with time; and (4) a less disturbed system would show a stronger connection between soil organisms and soil processes (i.e. soil respiration in our case).

2. Materials And Methods

2.1. Site description and experimental design

We performed this study at the long-term experimental field station (Systèmes d'Observation et d'Expérimentation pour la Recherche en Environnement Agrosystèmes Cycles Biogéochimiques et Biodiversité) 'arable crops', located at Estrées-Mons, Northern France (49.873 N, 3.032 E). The mean annual temperature is 10.8 °C, and the mean annual precipitation is 678 mm. The soil is classified as Luvisol (IUSS Working Group WRB. 2007). The physical, chemical and biological soil properties and crop rotations have been fully described in previous papers (Coudrain et al., 2016; Coulibaly et al. 2017).

Briefly, before this experiment, we applied the same crop (wheat in 2008, then barley in 2009) and the same conventional management to homogenize the soil. In 2010, a six-year rotation was initiated, composed of spring pea (*Pisum sativum*, L.), winter wheat (*Triticum aestivum*, L.), rapeseed (*Brassica napus*, L.), spring barley (*Hordeum vulgare*, L.), maize (*Zea mays*, L.) and again winter wheat. Alternatively, a perennial and bioenergy crop of switchgrass (*Panicum virgatum*, L.) was also planted. Six treatments were set up, with 4 replicates within a randomized block design. Five of these treatments, a total of 20 plots, were investigated in the present study: conventional tillage (CT), conventional tillage and reduced N fertilization (CT-RN), reduced tillage (RT), reduced tillage and residue removal (RT-RR), and perennial bioenergy crop (P-BC). In the RT-RR and P-BC treatments, crop residues were removed after harvest, while in the other treatments, crop residues were incorporated in soil by ploughing. Thus, over the rotation time, the mean amounts of carbon retained in the RT-RR and P-BC treatments were 1.6 t carbon ha^{-1} yr^{-1}, while those in the other treatments were 3.2 t carbon ha^{-1} yr^{-1}. For detailed information, see Table 1.

2.2. Soil and sampling

Soil samples were collected in spring 2012, 2013, 2014 and 2020, at the start of the vegetation season. Sampling in early spring allows researchers to catch the impact of former crop residue addition and soil tillage of the previous year, without having too large an impact of crop growth and rhizodeposition of the new cropping period. Steel cylinders (5 cm diameter, depth 5 cm) were used for sampling each year in each of the 20 plots. Each sampling year, five cores were taken from each plot, carefully mixed and then transported to the laboratory in cool boxes, and stored at 4 °C for microbial biomass, enzyme activity and nematode community analysis.

2.3. Soil microbial biomass, heterotrophic respiration, and enzymatic activities

Microbial biomass carbon (MBC) was determined using the fumigation-extraction method described by Vance et al. (1987). The extraction of dissolved organic C (DOC) from soil samples, either fumigated with chloroform or not, was performed with 40 mL of K_2SO_4 0.3 M (soil-solution 1:4, agitation 30 min, 20°C) and the concentration of DOC in extracts was analysed with a C-analyser (1010, O.I. analytical, Globalspec, NY, USA). The amount of C in microbial biomass was calculated as the difference in DOC contents between fumigated and unfumigated samples, with a correction factor (Jenkinson et al. 2004). C mineralisation was measured in standardised conditions using C-CO$_2$ emitted from soils during a 72-h incubation at 15°C, and was used as a proxy of soil heterotrophic microbial activity. For that purpose, moist soil samples (42 g equivalent dry soil at 18.6 g H$_2$O 100 g^{-1} soil) were placed in jars containing a beaker with water and a vial with 0.25 M NaOH solution to trap the CO$_2$ produced. The concentration of CO$_2$ trapped in the NaOH solution was measured by continuous flow colorimetry (Chaussod et al., 1986) using an autoanalyser (TRAACS 2000, Bran and Luebbe, Norderstedt, Germany).

Finally, enzyme activities involved in C, N, S and P cycles such as β-Glucosidase, Urease, Arylsulfatase, and Phosphatase were quantified using colorimetric method according to the ISO standard (ISO:20130:2018; Cheviron et al., 2022). All measurements were performed at the soil pH, in an unbuffered soil water solution. Soil enzymatic activities were expressed in mU.g^{-1} of dry soil corresponding to nmol of hydrolyzed substrate per minute for a gram of dry soil.

2.5. Soil nematode community analysis

Nematodes were extracted from 300 g fresh soils using the Oostenbrink elutriation method. On average, 185 nematodes per mass slide were identified to the genus level at 400 magnification (Bongers, 1988). We classified them into four trophic groups: bacterivores, fungivores, omnivores-predators and herbivores (Yeates et al., 1993).

We calculated nematode Shannon-Weaver diversity indices (H') and Simpson dominance indices (λ) as follows: H' = -∑pi(lnpi) and λ = ∑pi^2, where pi is the proportion of individuals in the ith taxon (Yeates and Bongers, 1999). Evenness indices were calculated as J = H'/ln S, where S is the total number of nematode genera in the community. We also calculated the nematode maturity indices (MI) and plant parasite indices (PPI) as follows: MI = ∑v(i)cp1-5 × ƒ(i)cp1-5, PPI = ∑v(i)cp2-5 × ƒ(i)cp2-5, where v(i) is the cp value of taxon i, and ƒ(i) is the proportion of that taxon of the total number of nematodes in a sample, and the value of cp1-5 are nematode functional guilds (Bongers, 1990). Enrichment indices (EI) and structure indices (SI) were also calculated as in Ferris et al. (2001), where a high EI value indicates that the soil is enriched, and a high SI value indicates the soil food web is stable. The nematode channel ratio (NCR) explains the relative contributions of the channels and is calculated as NCR = B/(B + F), where B and F are the relative densities of bacterivores and fungivores, respectively, to the total nematode density. The NCR is constrained to values between 1 (totally bacteria-mediated) and 0 (totally fungus-mediated) (Ferris and Matute, 2003).

2.7. Statistical analyses

Repeated measure ANOVAs were employed to determine the effects of the factor "Treatment" on nematode density, diversity and ecological indices, enzyme activity, MBC and soil heterotrophic respiration. Tukey tests were used to locate significant differences between treatments. The principal response curves (PRC) method was used to determine the temporal trends of soil nematode community composition (represented by both trophic groups and genus) for each treatment using CANOCO 4.5 (Ithaca, NY, USA). PRC is conducted by redundancy analysis (RDA). The result is a diagram showing the first principal component of the variance, explained by treatment on the y-axis along the sampling periods on the x-axis. The control treatment (conventional tillage in our case) is treated as a zero baseline (the horizontal line). The treatment effect is represented by the deviation of each fluctuating line from the zero baseline through time (Lepš & Šmilauer, 2003). Structural equation modelling (SEM) was used to test how soil respiration was affected by soil microbes and nematode trophic group density. A conceptual model (Fig. S1) was designed to show the basic trophic relationships among these predictors, and several tests were conducted to assess model fit, i.e. the χ2-test, the Bentler-Bonnet normed fit indices (NFI), and the goodness-of-fit indices (GFI) using AMOS 7.0 software (Eisenhauer et al., 2015).

3. Results

3.1. Agricultural treatments and nematode community

When compared with CT (conventional tillage), RT (reduced tillage) treatment tended to increased total nematode density (Table 3, F = 3.919, P = 0.095), P-BC (perennial biomass crop) treatment significantly decreased total nematode density (Fig. 1a). No difference was found regarding the total nematode density in RT-RR (reduced tillage and residue removal) and RT treatments. P-BC treatment significantly decreased absolute herbivore density compared to CT treatment (Fig. 1b), while the herbivore density did not differ between RT-RR and RT treatments (Table 3, F = 0.001, P = 0.971). When compared with CT, both CT-RN (conventional tillage and reduced N fertilization) and RT treatments significantly increased bacterivore and fungivore density (Fig. 1cd). The bacterivore and fungivore density in RT-RR treatment were significantly lower than in RT treatment (Table 3, F = 16.056, P = 0.007; F = 21.175, P = 0.004, respectively). RT treatment significantly increased omnivore-predator density compared to CT treatment (Fig. 1e). The omnivore-predator density in RT-RR treatment was significantly lower than in RT treatment (Table 3, F = 10.185, P = 0.019).

When compared to CT, repeated measure ANOVAs did not show significant treatment effect on either Shannon-Wiener indices, Pielou evenness indices, or Simpson dominance indices (Table 2). However, the Shannon-Wiener indices in RT-RR treatment were significantly lower than in RT treatment (F = 6.272, P = 0.046). In our five treatments, no differences in the enrichment indices and nematode channel ratio were found (Fig. 2ac). The structure and maturity indices were significantly higher in P-BC treatment than in CT treatment (Fig. 2bd), while the plant parasite indices and PPI/MI were significantly lower in P-BC treatment than in CT treatment (Fig. 2ef). No differences in the enrichment indices, structure indices, nematode channel ratio, plant parasite indices, maturity indices and PPI/MI were found in RT-RR and RT treatment. (Table 4).

The temporal dynamics of soil nematode functional guilds under four agroecology practices were similar (Fig. 3a). Specifically, compared to the control CT, the effect of CT-RN treatment increased the contribution of Ba$_2$ (F = 85.928, P = 0.000) and Fu$_2$ (F = 9.592, P = 0.021), while it decreased the contribution of He$_2$ (F = 49.429, P = 0.000) and He$_3$ (F = 7.575, P = 0.033). RT significantly (F = 30.145, P = 0.002) increased the contribution of Ba$_2$ and decreased the contribution of He$_3$ (F = 10.084, P = 0.019), as did P-BC treatment (F = 19.338, P = 0.005). The nematode functional guilds showed apparent successions from communities dominated by He$_3$ and He$_2$ to communities dominated by Ba$_2$ and Fu$_2$ after N and tillage were reduced (Fig. 3a).

Principal response curves demonstrated that the four agroecology practices affected the successions of soil nematode genus (Fig. 3b) during the course of the experiment. Specifically, when compared with CT, CT-RN and RT treatments significantly (F = 32.944, P = 0.001; F = 26.189, P = 0.002) increased the contribution of *Cephalobus* in nematode communities, while CT-RN and RT significantly (F = 8.248, P = 0.028; F = 13.106, P = 0.011) decreased the contribution of *Meloidogyne*. When compared with CT, CT-RN and P-BC significantly (F = 9.638, P = 0.021; F = 40.434, P = 0.001) decreased the contribution of *Paratylenchus* sp2. Alternatively, compared with CT, P-BC significantly (F = 21.079, P = 0.004) increased the contribution of *Psilenchus*, but significantly (F = 20.215, P = 0.004) decreased the contribution of *Meloidogyne*.

3.2. Different agricultural treatments on enzyme activity, microbial biomass and respiration

When compared with CT, CT-RN, RT and P-BC significantly increased soil arylsulfatase, glucosidase, phosphatase and urease activities (Fig. 4). When compared with RT, RT-RR significantly (F = 14.901, P = 0.008; F = 60.076, P = 0.000) decreased soil glucosidase and urease activities, and tended to decrease (F = 4.76, P = 0.072; F = 5.597, P = 0.056) arylsulfatase and phosphatase activity (Fig. 4). The CT-RN, RT and P-BC treatments had higher MBC than CT (Fig. 5a).

When compared with RT, RT-RR significantly (F = 185.83, *P* = 0.000) decreased soil MBC (Fig. 5a). CT-RN significantly stimulated soil respiration compared to CT (Fig. 5b) and RT-RR significantly (F = 6.359, *P* = 0.045) decreased soil respiration (Fig. 5b) when compared to RT.

3.3. Relationships between soil food web and respiration

Structural equation modelling showed that each agroecological practice led to different relationships, and thus paths, between MBC and soil respiration (Fig. 6). In RT treatment, the model was not significant, while the three other models (CT-RN, RT-RR and P-BC) were (Fig. 6a-d). Within the CT-RN treatment, MBC had positive correlation with bacterivores and fungivores, as well as with soil heterotrophic respiration. Bacterivores had positive correlation with omnivores-predators. Within the RT-RR treatment, MBC and bacterivores had positive correlation with soil respiration, while fungivores had negative correlation with soil respiration (Fig. 6c). In the P-BC treatment, MBC had negative correlation with fungivores and bacterivores. Omnivores-predators had positively correlation with soil respiration. Finally, herbivores had negative correlations with soil respiration in all the treatments (Fig. 6).

4. Discussion

4.1. Effects of reduced N fertilization

A meta-analysis suggested that high fertilizer N inputs simplify the nematode community structure and functions (Liu et al., 2016). Thus, we hypothesised that reduced N fertilization would increase maturity and structure indices. In contrast to our first hypothesis, we observed that the nematode ecological indices did not differ. It takes a long time to restore the nematode community structure and ecological indices in farmland, and this may depend on organic matter content. The higher the soil organic matter content, the faster the nematodes settle and mature. However, reduced N fertilization significantly increased absolute bacterivore and fungivore densities. Thus, the absolute densities of trophic groups were more responsive to reduced N than the relative densities of functional guilds on which the indices of maturity and enrichment are based. The higher density of bacterivores and fungivores with reduced N may have been mainly due to the parallel increase in microbial biomass, as confirmed by the structural equation modelling. When compared with CT, the increase in microbial biomass (+ 42%, + 71% and + 46% in 2012, 2013 and 2014, respectively) following a reduction of N supply is in line with many studies showing that N addition generally suppresses microbial biomass (see Wang et al., 2018) due to a combination of different factors, like soil acidification and leaching of major elements such as magnesium and calcium (Vitousek et al., 1997) and changes in osmotic balance. Alternatively, reduced N fertilization significantly increased the soil respiration, which was consistent with a previous study showing that excessive N can inhibit the activity of soil enzymes, reduce belowground carbon allocation and eventually cause reductions in soil respiration (Yan et al., 2021).

The nematode communities were previously shown to be regulated by resources (bottom-up controlled) (Neher, 2010). The higher microbial biomass carbon in CT-RN would then stimulate the microbial-feeding nematodes, and could result in a faster turnover of nutrients in soil, which could be confirmed by the higher enzyme activities. However, in this study, the nematode ratio channel was not affected by reduced N, but the high NRC values (above 0.5) indicated that bacterial decomposition pathways dominated and confirmed that bacterivores were more favoured than fungivores. Furthermore, across years, as confirmed by the principal response curves, the temporal trajectories of nematode community composition in CT-RN showed an increase of Ba_2 and Fu_2 functional guilds.

4.2. Effects of reduced tillage

Supporting our hypothesis, we found that reduced tillage (RT) was less harmful to nematodes than conventional tillage (CT), and tended to increase in their total density (*P* = 0.095). This is not surprising, as tillage is known to be rather destructive to soil food webs by not only disrupting the physical structure of soil, but by shifting the community to an earlier stage of ecological succession, with greater dominance of the bacterial than fungal pathway (Neher and Campbell, 1994; Treonis et al., 2010). Our results supported this finding, but alleviating tillage-linked-disturbance resulted, in our case, in an increase of both microbial feeders, namely bacterivores and fungivores. In contrast, it was shown that densities of both bacterial-feeding and fungal-feeding nematodes did not significantly change with tillage system, as indicated in a recent global meta-analysis (Puissant et al., 2021), even if contrasting local patterns did emerge. Reduced tillage significantly increased omnivore-predator density, mainly due to the higher microbial biomass in reduced tillage soil, which stimulated the bacterivores and omnivore-predators (Fig. 6). Alternatively, reduced tillage might have increased the stability of soil aggregates, and then increased physical protection to omnivore-predators (Martin and Sprunger, 2021). However, the general increase in density that we observed was not paralleled by an increase in diversity indices, structure indices or maturity indices in RT compared to CT, as we had postulated. It seems that, in our case, reducing tillage did not lead to more stable nematodes communities, or a micro-food web composed by persistent taxa, as could have been expected (Bongers, 1999, Villenave et al., 2018). As a matter of fact, the density of omnivore-predators, even if it increased, remained too low at the end of the trial to generate a significant increase in the structure indices, as the density of Ba_2 also increased. Over time, RT significantly increased the contribution of the Ba_2 functional group, with an increase of *Cephalobus*. In parallel a decrease of the herbivore *Meloidogyne* was also noticeable.

4.3. Effects of crop residues removal

Supporting our hypothesis, crop residue removal negatively affected soil nematode density with only herbivores not being affected (RT-RR vs RT). Alpha diversity of soil nematodes was also lower after crop residue removal, as was soil microbial biomass. Retaining crop residues is known to result in a higher microbial biomass, because crop residues serve as an energy source for soil microorganisms (Govaerts et al., 2007). Through a bottom-up control, nematodes benefit from this increase in basal resources, allowing for a higher diversity through resource partitioning. Alternatively, retaining crop residues improves soil chemical and physical conditions, such as aggregate stability, penetration resistance and surface slaking (Govaerts et al., 2006). However, crop residue retention is not always favourable for decomposers. In the same study site, the higher decomposer group (e.g. Collembola) did not respond to the residue removal (Coulibaly et al., 2017), possibly due to the mechanical disturbance resulting from the superficial burying of residuals with disk ploughing, in combination with an increase of top-down regulation imposed by predators favoured by the new topsoil habitat conditions provided by crop residues. When it

comes to micro-food webs or basal trophic levels, it seems clear that removing crop residues is unfavourable and pauperizes the communities. As a consequence of decreases in nematode communities and microbial biomass, soil respiration also decreased in RT-RR compared to RT.

4.4 Effect of perennial crop

Supporting our hypothesis, SI and MI clearly increased in P-BC treatment during the course of the experiment, in contrast to the other practices. P-BC, the most conservative practice in our case and one that did not involve soil disturbance, led to a more stable micro-food web (high maturity and structure indices). Our results support the findings that established perennial crops support more highly structured and complex food webs relative to annual cropping systems (Chauvat et al., 2014, Coulibaly et al., 2017, Sprunger et al., 2019), through a stimulation of the microbial system mostly due to i) inputs above- or belowground of fresh organic material or easily assimilable rhizodeposits (Sun et al., 2019) and ii) buffering of microclimatic conditions (Kim et al., 2022). This could also be confirmed by the higher enzyme activities. We also hypothesised that perennial crops would increase nematode density. However, P-BC treatment did not have significant effects on bacterivore and fungivore density. This might have been due to their being regulated through the top-down role imposed by omnivore-predators. P-BC treatment significantly decreased herbivore density, which was favourable for root growth. Thus, perennial crops are beneficial to many soil functions in agroecosystems (Zhang et al., 2022).

4.5 Temporal trends of different agricultural practices

Alpha diversity indices seem poor predictors of practices' effects in comparison to nematode indices, with the taxonomic diversity of the nematode community being unaffected even after 5 years. As all the plots were under conventional tillage prior to our experiment, it seems that at least 4 to 5 years are necessary for nematode genera from the surrounding landscape to colonise plots that have been converted to different management. Free-living nematodes, due to their small size, are known to be poor active dispersers, especially in a large agricultural landscape matrix like the one we investigated, although they can be easily passively dispersed by wind or water runoff. Therefore, profound changes in community composition due to colonisation might take at least 4 to 5 years to occur (Villenave et al., 2018).

However, changes in guilds dominance were already detectable 2 years after treatment establishment, with a strong interannual variation in trophic groups. We also observed variations between years in functional guilds, resulting in significant shift in EI and especially SI (EI exhibited significant but still low variations, between 30 and 50%). Furthermore, specific functions like enzymatic activities or soil respiration were affected by the interactions between the factors 'year' and 'practices', and closely related to microbial C biomass and nematodes.

All enzymatic activities followed the same pattern, with a clear differentiation across time and constantly lower values in CT and RT-RR, which seemed to be the less favourable practices for soil biodiversity and associated functions. The residue removal in RT-RR might lead to a lower amount of fresh soil organic matter combined with a high level of fertilization, and these are probably the key factors diminishing microbial enzymatic activities, especially bacterial ones. According to our SEM results, it is clear that overall nematodes are closely associated to microbial biomass and soil respiration. Nematodes were already shown to drive microbial communities, their functions and, indirectly, plant performances as well (see Wilschut & Geisen, 2021). Interestingly, the connections between nematodes and microbial biomass C differed according to the agricultural practices, but there were still consistent linkages, with, for example, a negative correlation between the density of herbivores and the soil respiration or positive correlation between bacterivores and omnivores/predators. This was true even if inter-annual variations were quite important in our dataset. Such linkages may either be direct or indirect; it is still unclear, from a mechanistic point of view, how changes in agricultural practices and level of soil system disturbance might modify relationships between groups, or between groups and processes. However, several factors might come into play. For example, by filtering out particular traits of a trophic group, soil disturbance might lead to disconnections between trophic levels. This traits-dismatching between trophic groups, already noticed in a different context of soil disturbance (Brousseau et al., 2021) may have potential consequences for soil functions. Furthermore, functional consequences of traits filtering were also demonstrated in the literature (Wolf et al., 2021).

To conclude, our results suggest that converting conventional agricultural practices towards agroecological ones modify not only belowground nematofauna, but also connections between trophic groups and associated processes. While reducing disturbances like fertilization or tillage allow the development of a more mature and structured nematode community, interannual variation due to climatic conditions seems to be a strong driver too. Only a multi-year follow-up of practices can provide reasonable estimates of modifications of the soil micro-food web taking place after changes in agricultural practices, which has been too rarely done in the literature so far.

Declarations

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Tables

Table 1 Description of the 5 experimental treatments of the experimental field-station SOERE ACBB located in Estrées-Mons, France, according to three main practices: tillage depth, crop residue management, and N fertilization rate.

Treatment	Tillage depth (cm)	Crop residues management	Nitrogen fertilization rate (kg nitrogen ha^{-1} yr^{-1})
conventional tillage (CT)	25	Crop residue retained (3.2 t carbon ha^{-1} yr^{-1})	134
conventional tillage and reduced N fertilization (CT-RN)	25	Crop residue retained (3.2 t carbon ha^{-1} yr^{-1})	43
reduced tillage (RT)	8	Crop residue retained (3.2 t carbon ha^{-1} yr^{-1})	134
reduced tillage and residues removal (RT-RR)	8	Crop residue removed (1.6 t carbon ha^{-1} yr^{-1})	134
perennial and bioenergy crop (P-BC)	25	Crop residue removed (1.6 t carbon ha^{-1} yr^{-1})	134

Table 2 Alpha diversity indices of soil nematode communities under five different agricultural treatments in three sampling years (2012, 2013 and 2014). CT: Conventional tillage, CT-RN: Conventional tillage and nitrogen reduced, RT: Reduced tillage, RT-RR: Reduced tillage and crop residues removal, P-BC: Perennial and bioenergy crop. H', J and λ stand for Shannon-Wiener diversity indices, Pielou evenness indices, and Simpson dominance indices. Mean ± standard error of means.

	2012					2013					2014
Index	CT	CT-RN	RT	RT-RR	P-BC	CT	CT-RN	RT	RT-RR	P-BC	CT
H'	1.89±0.18	2.04±0.15	2.21±0.04	2.16±0.06	2.07±0.08	2.24±0.05	2.34±0.1	2.32±0.06	2.25±0.1	2.51±0.06	2.3±0.13
J	0.68±0.06	0.75±0.03	0.78±0.02	0.76±0.02	0.74±0.03	0.75±0.01	0.77±0.03	0.76±0.03	0.75±0.03	0.79±0.01	0.78±0.01
λ	0.26±0.07	0.19±0.04	0.15±0.01	0.16±0.01	0.19±0.02	0.16±0.01	0.14±0.02	0.15±0.01	0.16±0.02	0.13±0.01	0.14±0.02

Table 3 Results of repeated measure ANOVAs on nematode density. Tukey tests were used to locate significant differences between treatments.
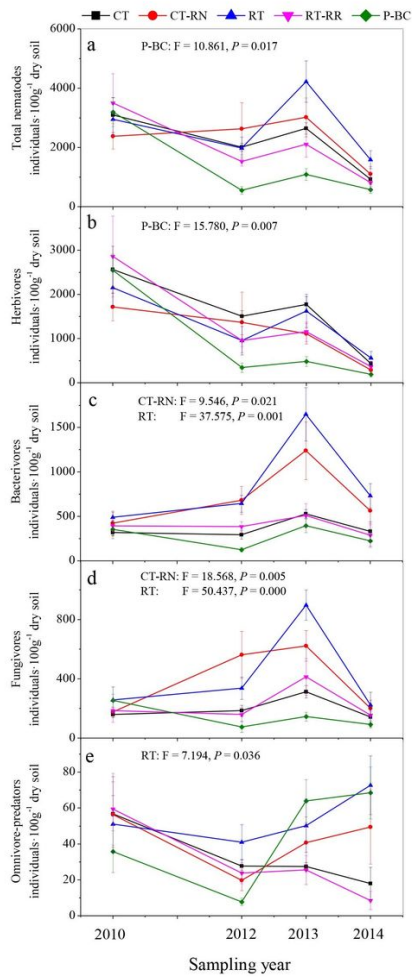
	Total nematodes		Herbivores		Bacterivores		Fungivores		Omnivore-predators	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
CT and CT-RN	0.077	0.790	2.637	0.156	**9.546**	**0.021**	**18.568**	**0.005**	0.612	0.464
CT and RT	3.919	0.095	1.103	0.334	**37.575**	**0.001**	**50.437**	**0.000**	7.194	**0.036**
CT and RT-RR	0.166	0.698	0.574	0.477	0.061	0.814	0.327	0.588	0.111	0.750
CT and P-BC	**10.816**	**0.017**	**15.780**	**0.007**	2.931	0.138	2.414	0.171	1.481	0.269
RT and RT-RR	2.308	0.180	0.001	0.971	**16.056**	**0.007**	**21.175**	**0.004**	**10.185**	**0.019**

Table 4 Results of repeated measure ANOVAs on nematode indices. Tukey tests were used to locate significant differences between treatments. Nematode channel ratio: NCR. Plant parasite indices: PPI. Maturity indices: MI.

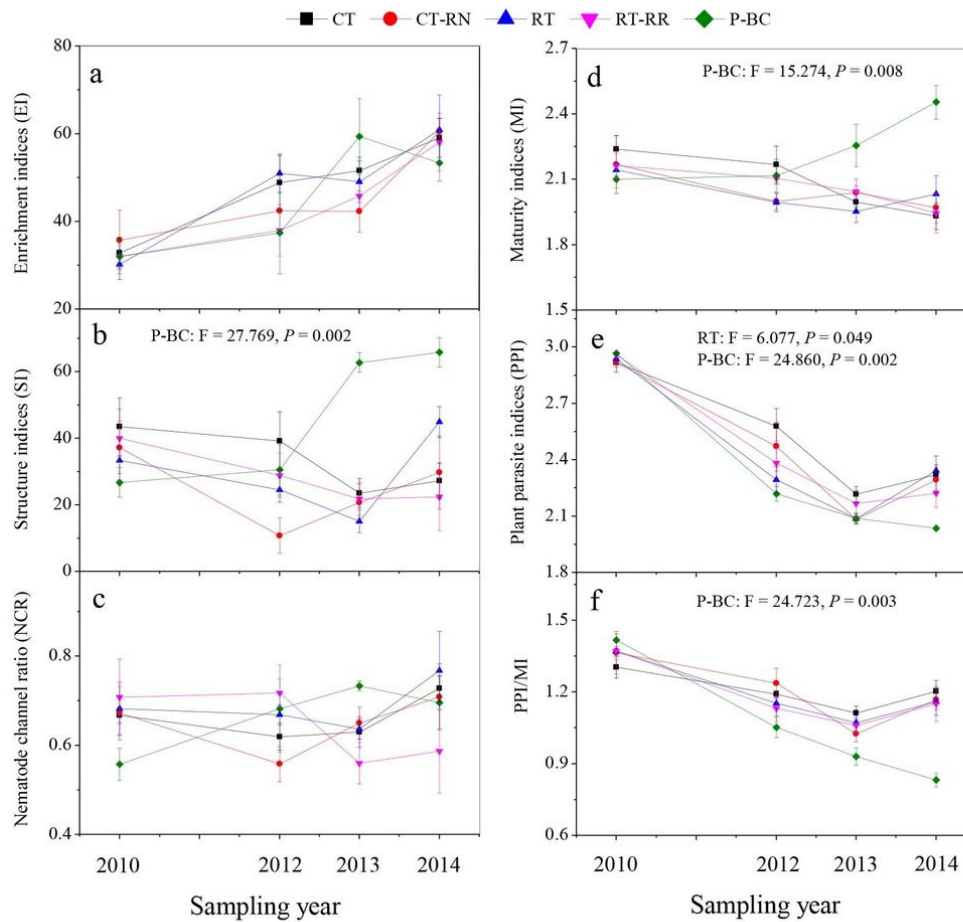|	Enrichment indices		Structure indices		NCR		Maturity indices		PPI		PPI/MI	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
CT and CT-RN	1.359	0.288	3.408	0.114	0.326	0.589	1.055	0.344	3.677	0.104	0.018	0.896
CT and RT	0.018	0.899	2.910	0.139	0.516	0.500	1.963	0.211	**6.077**	**0.049**	0.178	0.688
CT and RT-RR	2.329	0.178	3.088	0.129	0.354	0.574	0.338	0.582	1.327	0.293	0.328	0.588
CT and P-BC	1.006	0.355	**27.769**	**0.002**	0.045	0.839	**15.274**	**0.008**	**24.86**	**0.002**	**24.723**	**0.003**
RT and RT-RR	2.024	0.205	0.403	0.549	1.149	0.325	1.283	0.300	0.085	0.780	0.081	0.785

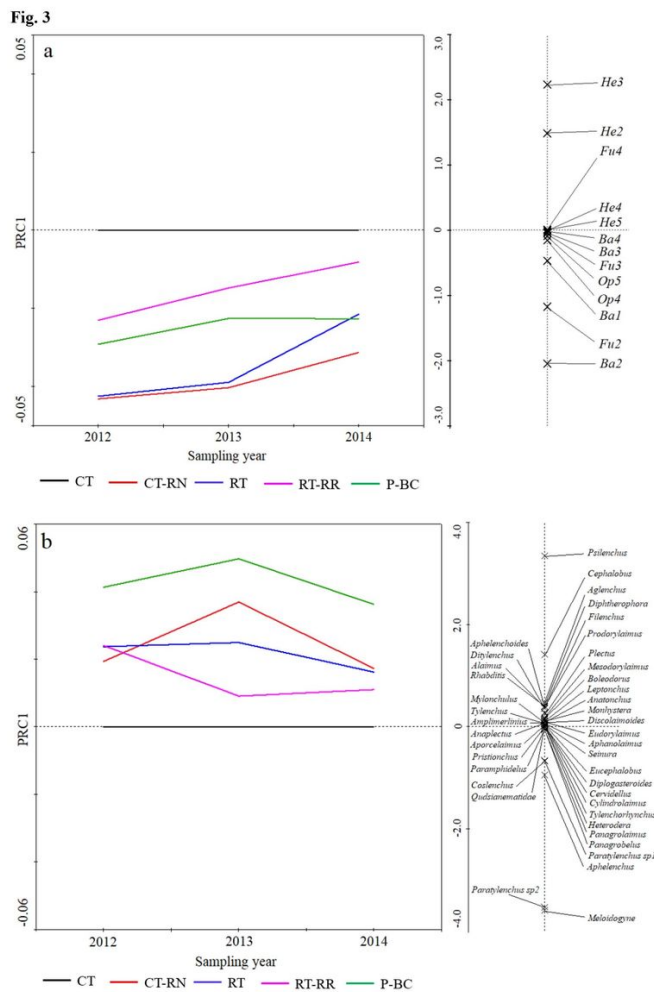# Figures

Fig. 1

Figure 1

Densities of total nematodes (a), herbivores (b), bacterivores (c), fungivores (d), and omnivore-predators (e) under five different agricultural treatments in each sampling event (2020, 2012, 2013 and 2014). CT: Conventional tillage, CT-RN: Conventional tillage and nitrogen reduced, RT: Reduced tillage, RT-RR: Reduced tillage and crop residues removal, P-BC: Perennial and bioenergy crop. Bars indicate standard errors of means. Only significant ($P < 0.05$) effects of treatments compared to conventional tillage (CT) found with repeated measure ANOVAs and Tukey tests are shown on the graphs.

Fig. 2

Figure 2

Enrichment indices (a), structure indices (b), nematode channel ratio (c) and maturity indices (MI) (d) under five different agricultural treatments in each sampling event (2020, 2012, 2013 and 2014). CT: Conventional tillage, CT-RN: Conventional tillage and nitrogen reduced, RT: Reduced tillage, RT-RR: Reduced tillage and crop residues removal, P-BC: Perennial and bioenergy crop. Bars indicate standard errors of means. Only significant ($P < 0.05$) effects of treatments compared to conventional tillage (CT) found with repeated measure ANOVAs and Tukey tests are shown on the graphs.
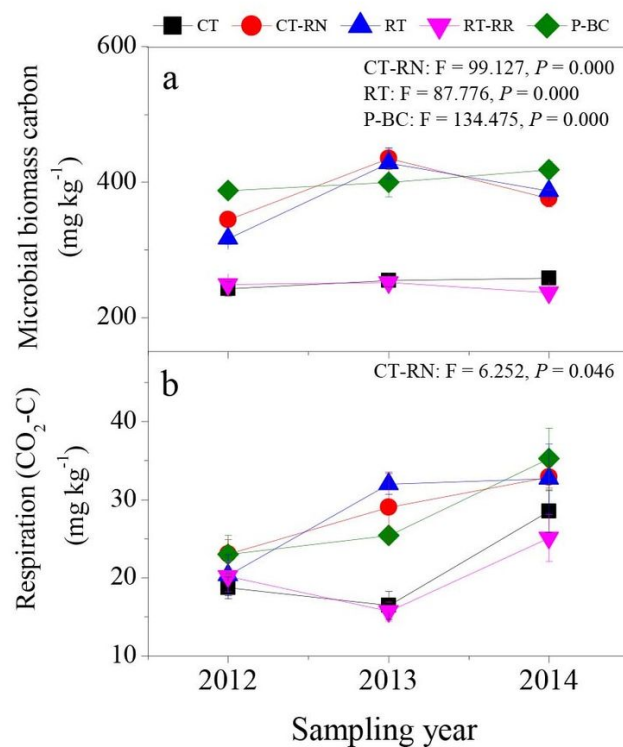
Figure 3

Principal response curves (PRC) with weights of density of each soil nematode functional guild (a) and nematode genus (relative abundance > 1%) (b) under five different agricultural treatments in each sampling event (2012, 2013 and 2014). CT: Conventional tillage, CT-RN: Conventional tillage and nitrogen reduced, RT: Reduced tillage, RT-RR: Reduced tillage and crop residues removal, P-BC: Perennial and bioenergy crop. The horizontal axis represents the control treatment. He, herbivores, Ba, bacterivores; Fu, fungivores; Om, omnivore-predators.

Fig. 4
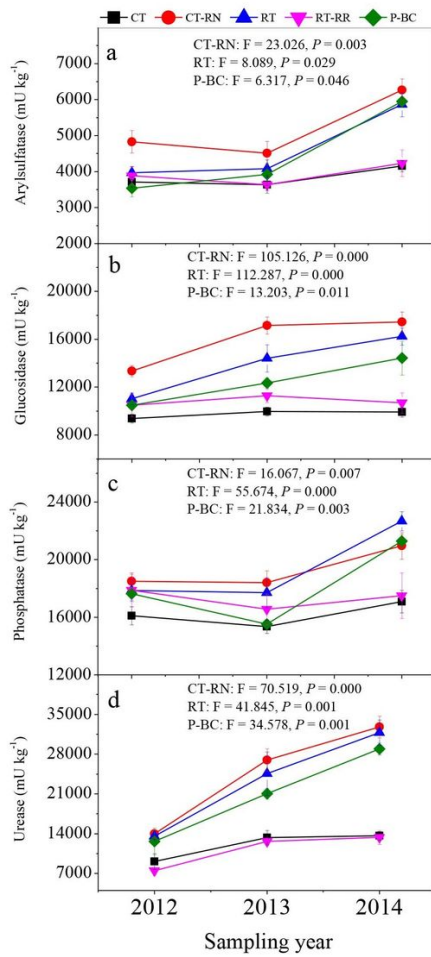
Figure 4

Soil enzyme activities under five different agricultural treatments in each sampling event (2012, 2013 and 2014). CT: Conventional tillage, CT-RN: Conventional tillage and nitrogen reduced, RT: Reduced tillage, RT-RR: Reduced tillage and crop residues removal, P-BC: Perennial and bioenergy crop. Bars indicate standard errors of means. Only significant ($P < 0.05$) effects of treatments compared to conventional tillage (CT) found with repeated measure ANOVAs and Tukey tests are shown on the graphs.

Fig. 5

Figure 5

Soil microbial biomass carbon (a) and respiration (b) under five different agricultural treatments in each sampling event (2012, 2013 and 2014). CT: Conventional tillage, CT-RN: Conventional tillage and nitrogen reduced, RT: Reduced tillage, RT-RR: Reduced tillage and crop residues removal, P-BC: Perennial and bioenergy crop. Bars indicate standard errors of means. Only significant ($P < 0.05$) effects of treatments compared to conventional tillage (CT) found with repeated measure ANOVAs and Tukey tests are shown on the graphs.

Fig. 6
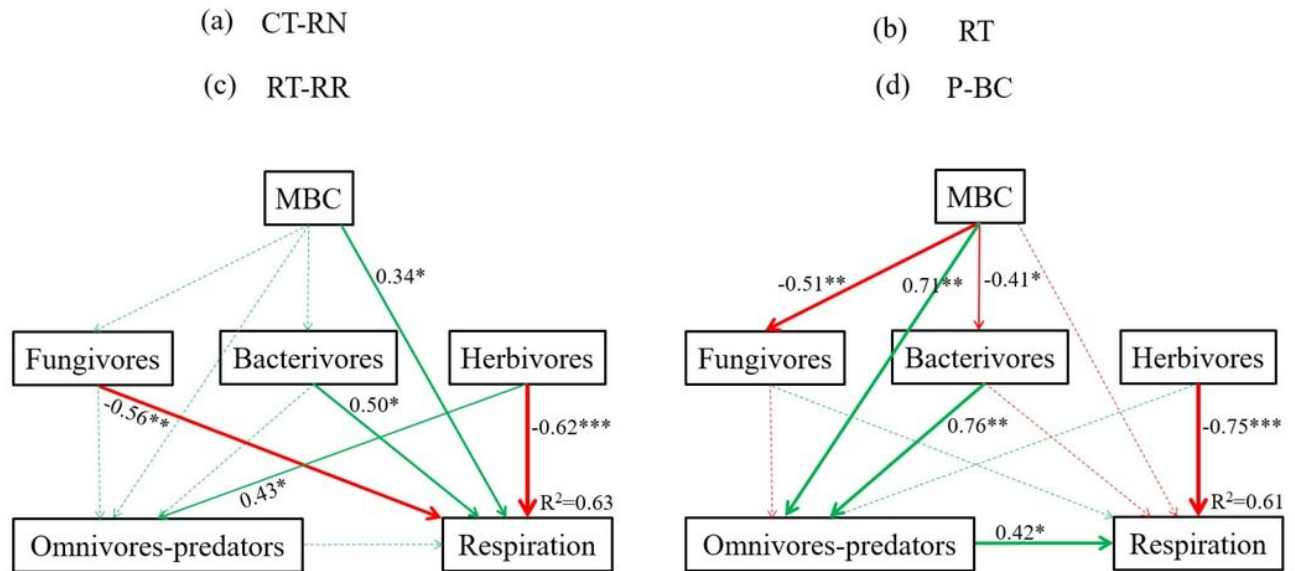
Figure 6

Structural equation model relating soil respiration to the treatments of conventional tillage and reduced nitrogen (a), reduced tillage (b), reduced tillage and residue removal (c) and perennial and bioenergy crop (d). Numbers on arrows are standardized regression coefficients. (a) $\chi2 = 5.723$, $P = 0.057$, NFI = 0.930, GFI = 0.932, RMSEA = 0.284. (b) $\chi2 = 9.532$, $P = 0.023$, NFI = 0.911, GFI = 0.898, RMSEA = 0.308. (c) $\chi2 = 4.357$, $P = 0.225$, NFI = 0.927, GFI = 0.946, RMSEA = 0.140. (d) $\chi2 = 1.714$, $P = 0.191$, NFI = 0.981, GFI = 0.977, RMSEA = 0.176. Arrows represent positive (green solid line), negative (red solid line) and non-significant (dashed line) path coefficients. The thickness represents the magnitude of the path coefficients. (*) $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Supplementary Files

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