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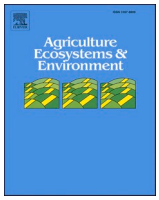

Soil extracellular enzyme activity increases during the transition from conventional to organic farming

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ABSTRACT

There is an increasing interest in developing agricultural management practices that support a more nature-based, sustainable food production system. In organic systems, extracellular enzymes released by soil microorganisms are important regulators of the cycling and bioavailability of plant nutrients due to the lack of synthetical inputs. We used a chronosequence coupled with a paired field approach to evaluate how potential activity of hydrolytic soil extracellular enzymes changed over time (0–69 years) during the transition from conventional to organic agriculture in two types of soils, marine clay and sandy soils. Organic management generally enhanced the activity of enzymes related to the C cycle, particularly in sandy soils, and increased the proportion of C-related enzymes relative to N- and P-related enzymes. Differences in soil extracellular enzyme activity between organic and conventional farming increased with time since conversion to organic farming for α-β-glucosidase, xylosidase, phosphomonoesterase, 4-N-acetylglucosaminidase, arylsulphatase, and the ratio of C:N enzymes. In some cases, the divergence in enzyme activity was driven by enhanced activity with time in organic fields, but in others by reduced activity over time in conventional fields. Our findings suggest that organically managed soils with higher enzyme activity may have a greater potential for organic matter breakdown, residue decomposition, and higher rates of cycling of C and nutrients. However, these positive effects may take time to become apparent due to legacy effects of conventional management.

1. Introduction

Approximately 38 % of global land in the world is used for agriculture (FAO, 2020). Since 1960, high rates of synthetic fertilizers and biocides have been applied to agricultural soils to enhance their yield (Erisman et al., 2008). However, these agricultural production methods can have negative impacts on the environment, increasing leaching of nutrients, emission of greenhouse gases, and reducing above- and

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belowground biodiversity (de Vries et al., 2023; Wolters et al., 2000). Therefore, there is an increasing interest in developing agricultural management practices that support a more sustainable food production system (Struik and Kuyper, 2017). Applying nutrients in the form of organic amendments instead of synthetic fertilizers may enhance soil biodiversity (Martínez-García et al., 2018), thus switching to organic farming practices may be an important step towards improving the sustainability of agricultural systems (Struik and Kuyper, 2017). In organic farming systems, plants largely rely on nutrient supply via mineralization from soil organic matter, manure, compost, and other organic amendments (Reganold and Wachter, 2016). However, these organic inputs first need to be decomposed and mineralized so nutrients can become available to crops. Although soil extracellular enzyme activity is known to play a vital role in the decomposition and mineralization of organic substrates in soils by catalyzing the degradation of organic compounds of varying complexity (Sinsabaugh et al., 2009), relatively little is known on how, and how fast, soil enzyme activities respond to the conversion from conventional to organic agricultural management.

Soil extracellular enzyme activities have been widely proposed as a key indicator of soil quality given that they catalyze the transformation of minerals and complex organic compounds into more bioavailable nutrient forms (Dick, 1997). Extracellular enzymes in soils have a variety of origins such as plant roots, soil biota, including microorganisms like bacteria and fungi, and even necromass (Baldrian, 2014). All these enzymes are important for the functioning of ecosystems, being key regulators of nutrient cycling and bioavailability in agricultural soils (Puglisi et al., 2006; Stott et al., 2010). Moreover, stoichiometric ratios of enzymes linked to C:N:P cycling are considered as an indicator of relative resource availability and net immobilization/mobilization rates of nutrients in agroecosystems, and thus of microbial nutritional demands (Sinsabaugh et al., 2009). In other words, the expression of enzymes is the result of the regulation of cellular metabolism by environmental nutrient availability (Sinsabaugh et al., 2009). Thus, characterizing shifts in enzyme activity and C:N:P stoichiometric enzyme ratios during the transition from conventional to organic management can offer novel insights to understand how organic matter processing and nutrient supply and demand are affected over time due to changes in the management regime.

Previous studies have shown that land management is a main driver of soil enzyme activity. For example, intercropping, wider crop rotations, and reduced tillage may increase soil enzyme activity (Curtright and Tiemann, 2021; Liang et al., 2014; Mbuthia et al., 2015; Tiemann et al., 2015). In addition, soil extracellular enzyme activity is often higher in soils under organic arable farming than in those that are managed conventionally (Lori et al., 2017; Mäder et al., 2002). Moreover, increases in C:N and C:P enzyme ratios have been frequently linked to an enhanced input of organic amendments that often have high ratios of C to N and/or P (Ashraf et al., 2021). However, changes among agricultural management strategies may entail lag times from legacy effects of the previous management (García-Ruiz et al., 2008; Schrama et al., 2018). As a result, shifts in soil enzyme activity and enzyme ratios may not occur immediately upon conversion from conventional to organic farming. Generally, soil enzyme activity increases following agricultural land abandonment or when land is deliberately converted to non-agricultural ecosystems (Raiesi and Salek-Gilani, 2018; Waldrop et al., 2000). For example, using a chronosequence of grassland restoration sites, Yang et al. (2020) used soil extracellular enzyme stoichiometry to show that microorganisms were co-limited by N and P, and that N limitation was gradually exacerbated over time. This indicates that understanding temporal responses of enzyme activity and enzyme ratios may be key to understanding how soil functioning changes during land-use transitions. Evaluating how soil enzyme activity changes during the conversion from conventional to organic land management using long-term (e.g., supradecadal) chronosequences can offer valuable insights to better understand the temporal dimension of regime transitions.

The production and activity of soil extracellular enzymes depends on soil properties, including texture, soil organic matter (SOM), and pH (Sinsabaugh et al., 2008; Tabatabai, 1994). For example, sandy soils generally have lower microbial biomass, water retention capacity, and SOM content, resulting in less enzyme activity than in clay or silt soils (Gomez et al., 2020; Risch et al., 2019). In contrast, soils with higher clay and silt content usually have higher enzyme activity due to generally greater SOM content, microbial biomass, and minerals such as Mn and Co, essential for their catalytic activity (Bell et al., 2022; Burns et al., 2013). However, enzyme activity can also be reduced in clay soils due to long-term adsorption of enzymes by electrically charged clay particles (Burns et al., 2013). Knowing how soil properties (e.g., more clay vs. more sandy soils) determine the responses of soil enzyme activity during the transition from conventional to organic management will help to understand how changes in soil functioning differ among a wider range of agricultural soil types.

The main goal of this study was to evaluate how potential activity of soil extracellular enzymes changes over time during the transition from conventional to organic agriculture in marine clay and sandy soils. We used a chronosequence approach by collecting soil samples from arable farms across the Netherlands that had been converted from conventional to organic management between 0 and 69 years ago at the time of sampling. For each organic field, a local control was chosen by collecting soil samples from neighboring fields under conventional management with a similar type of crop. We measured the activity of soil extracellular enzymes in air-dried soils, as well as in soils that were revitalized by incubating them at 65 % of soil water-holding capacity and 22 °C for 40 days. We predicted that (i) enzyme activity would be greater under organic than conventional management, particularly in clay soils. We also hypothesized that (ii) C:N and C:P enzyme ratios would be higher under organic farming, and more so in clay versus sandy soils due to differences in organic matter content. Finally, we predicted that (iii) differences in soil extracellular enzyme activity and enzyme ratios between organic and conventionally managed soils would amplify with increasing time since conversion to organic farming.

2. Material and methods

2.1. Study sites

We collected soil samples from arable fields located in the Netherlands. Sites were established in a moderate maritime climate (Köppen type Cfb), with relatively mild winters and mild summers (Kottek et al., 2006). Mean annual temperatures range between 9.6 and 11.4 °C and precipitation is common throughout the year, averaging 800–975 mm. There were two soil types: (i) sandy soils, defined as Anthrosols with a very low elutriable fraction and an A-horizon of at least 30 cm; and (ii) marine clay soils, defined as Fluvisols from marine origin with an elutriable fraction of 17.5 %-45 %. Clay content varied between 1 % and 33 % (Table 1). Soil pH was determined using a pH meter after mixing 10 g of dry soil in 25 ml of demi water and allowing the mix to settle and stabilize. Soil pH ranged from 4.7 to 8.3 (Table 1). Soil organic matter (SOM) content was determined by loss-on-ignition. For this, samples were dried at 105°C and then placed in a muffle furnace for 8 h at 430°C. Soil organic matter content was calculated as the difference between samples heated at 105 and 430°C. Soil organic matter content ranged between 1.6 % and 8.3 % and was higher in sandy soils (Table 1). Soil clay content, pH, and organic matter content did not vary across managements, as was reported in van Rijssel et al. (2022).

To investigate how soil enzyme activity responds to conventional and organic management, and to time since conversion from conventional to organic management, we used a chronosequence of 74 arable fields with sandy and marine clay soils (van Rijssel et al., 2022). Half of the fields were under conventional management, and half under organic management. We used a paired approach by selecting organic fields of

Table 1
Summary of soil properties across management and soil types. SD = standard deviation. min = minimum. max = maximum. SOM = soil organic matter.

Management	Soil	Clay (%)				Sand (%)				pH				SOM (%)			
		mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
Conventional	Clay	19.96	5.57	8.00	33.00	35.34	10.92	14.00	59.00	7.99	0.22	6.91	8.25	3.31	0.92	1.62	5.43
Organic	Clay	19.51	5.78	10.00	30.00	36.61	12.72	12.00	63.00	7.96	0.12	7.66	8.20	3.49	0.92	1.82	6.29
Conventional	Sand	1.54	0.64	1.00	3.00	83.10	3.77	76.00	90.00	6.08	0.59	4.70	7.44	4.33	1.42	2.71	8.28
Organic	Sand	2.28	1.51	1.00	8.00	82.49	5.45	71.00	90.00	5.96	0.51	4.99	7.21	4.17	1.23	1.68	6.06

different conversion ages (between 0 and 69 years ago). For each organic field, we also collected soil from a nearby conventional field to serve as a local control. This allowed us to test for management and time impacts, while also controlling for local variation in edaphoclimatic conditions. Organic fields were selected according to the SKAL certificate ("Stichting Keur Alternatief voortgebrachte Landbouwproducten"), which is a Dutch certification for organic farms based on the European legislation (www.skal.nl). Requirements for obtaining a SKAL certification are that 70 % or more of the fertilizers are certified organic (animal manure, plant materials or compost), thereby minimizing the use of mineral fertilizers. In addition, there is no use of conventional chemical pesticides. Further information on the design of the chronosequence approach has been provided by van Rijssel et al. (2022).

Sites were selected according to: (1) soil type: either sandy or marine clay soils; (2) type of crop: we selected soils that were cultivated with either a cereal (53 out of 74 fields), including winter cereals like wheat (31 out of 74 fields) and spring cereals like barley (22 out of 74 fields), or a grass-legume mixture (21 out of 74 fields) containing clover (*Trifolium* sp.) or alfalfa (*Medicago sativa*); (3) rotation: fields needed to be under a crop rotation with tuber crops (e.g., potatoes, onions); and (4) ploughing: soils should have been ploughed with an inversion plough at least once in the last five years before sampling, as inverting the soil can have a major effect on soil biota and structure. During our sampling, we did our best to maintain the type of crop as comparable as possible. However, this was not possible at times, which resulted in a small experimental imbalance.

2.2. Soil sampling

All soil samples were collected during the early summer (between June - July) of 2017. In each field, we collected three subsamples separated by a minimum distance of 15 m. Each subsample was collected using an auger from a 2 m x 2 m area and contained approximately 3 kg of soil. For 74 fields, this resulted in a total of 222 individual soil samples that were processed and analyzed separately. Soil samples were taken at 5–15 cm depth. The top 5 cm was excluded to avoid the impact of variations in daily weather conditions (e.g., daily temperature, radiation received, frost, etc.). Soils were collected within the interior of the fields to avoid edge effect and tractor tracks. Samples were not analyzed fresh because of organizational constraints during the sampling campaign. Instead, once in the lab, soil samples were air-dried at room temperature and then stored at 4°C until further processing, which also allowed us to homogenize soil conditions at the time of analyses.

2.3. Soil extracellular enzyme activity

Before determining extracellular enzyme activities, large macroaggregates were gently broken down manually with a mortar, and large roots, stones, and shells were removed. Enzyme assays were carried out both in air-dried soils and revitalized soils. To revitalize the soil microbial community, we incubated air-dried soil samples (15 g) in 50 ml propylene falcon tubes loosely screwed for 40 days. Soils were kept at 22 °C and were adjusted to 65 % water-holding capacity prior to incubation. Soil moisture was readjusted twice during the incubation period based on mass loss. By incubating the soils, we were able to measure the production of hydrolytic enzymes from dormant microorganisms that were reactivated due to the presence of water (Allison and Vitousek, 2004; Blagodatskaya and Kuzyakov, 2013; Nannipieri et al., 1983). This approach has been used by others to enable comparisons of soil enzyme activities on a standardized basis by ensuring the same soil moisture content and temperature conditions for soils sampled across a diversity of sites (Blagodatskaya et al., 2016).

We used a high-throughput fluorometric approach to assay soil enzyme activities (Bell et al., 2013). We measured the potential activity of eight hydrolytic soil enzymes, including four enzymes related to the C cycle (α-1,4- glucosidase [AG], β-1, 4-glucosidase [BG], β-D-cellobiohydrolase, [CB], β-xylosidase [XYL]), two enzymes related to the N cycle (β-1, 4-N-acetylglucosaminidase [NAG]; leucine aminopeptidase [LAP]), one enzyme related to the P cycle (phosphomonoesterase [PHOS]), and one enzyme related to the sulfur (S)-cycle (arylsulphatase [AS]). Briefly, we incubated 1 g of air-dried soil in 30 ml of DI water. We used water instead of a buffer, as frequently done in other studies, because soils contain an array of buffering components that effectively control the pH within the soil environment (German et al., 2011; Li et al., 2021; Margenot et al., 2018). This approach has the advantage of reflecting soil sample-specific pH, which should better reflect *in situ* activities (Burns et al., 2013) while preserving differences among soil enzyme activities inherent to a soil (Wade et al., 2020). Samples were hoamogenized by vortexing for 10 seconds, and soil slurries were added into black 96-well plates. Soil slurries were incubated with a nonlimiting amount of fluorescently labeled (i.e. C-, N, or P-rich) substrates to enable the assay of enzyme activities at V_{max} (German et al., 2011). We used two synthetic fluorescent-based substrates: 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (MUC). MUC-linked substrates are used to assay the degradation of N-rich synthetic substrates such as proteins and/or amino acids (LAP), whereas MUB-linked substrates are used for the rest of hydrolytic enzymes. Slurries with fluorometric substrates were incubated for 1.5 h at 35 °C (Bell et al., 2013) and scanned on a microplate fluorometer reader (FTX-800, Biotek) to detect the fluorescence intensity of the released product (MUB or MUC) using excitation and emission wavelengths of 365 nm and 450 nm, respectively. Enzyme measurements were expressed in nmols of activity per g soil per hour. In addition to enzyme activity, we also calculated the stoichiometric ratios of enzymes in order to obtain information about nutrient demand as described in Sinsabaugh et al., (2009): ln(AG +BG + CBH + XYL):ln(NAG + LAP) (C:N acquisition); ln(BG +AG + CBH + XYL):ln(PHOS) (C:P acquisition); and ln(LAP + NAG):ln(PHOS) (N:P acquisition).

2.4. Data analysis

All analyses were done in R version 4.0.3. Significant differences were considered at $P < 0.05$. First, we used general linear mixed models to evaluate the effect of management (conventional vs. organic), soil type (marine clay vs. sandy), and their interactions on the activity of individual soil enzymes, as well as on the sum of the activities of enzymes related to C and N cycles (Sinsabaugh et al., 1992). These analyses were carried out separately for incubated vs. non-incubated samples. We used samples nested within fields, and fields within paired sites as a random factor. Additionally, we carried out linear mixed effects models using clay content as a covariate instead of soil type as a categorical variable. Moreover, given the different types of crops being considered,

we repeated these analyses but only considering sites that had cereals, which allowed us to reduce the noise generated by mixing crops. For these analyses, we used the *lme* function from the *nlme* package in R (Pinheiro et al., 2017).

We used linear mixed models to evaluate the impact of time since conversion on enzyme activity. Management and soil type were considered fixed factors and time since conversion effect was used as a co-variate. Conventional plots were assigned the same age as their neighboring organic plot. Therefore, we considered that there was a time effect when we found a significant management by time interaction because we did not expect changes in soil extracellular enzyme activity to become more distinct over time depending on management. Finally, we carried out Pearson correlations to evaluate the relationships between soil enzyme activity and soil properties (clay and sand content, organic matter content, and pH).

3. Results

Across the 74 arable fields evaluated, the potential activity of soil extracellular enzymes was highest for N-cycling enzymes, intermediate for P- and C-cycling enzymes, and lowest for S-cycling enzymes (Figure S1). In non-incubated samples, phosphomonoesterase and L-leucine aminopeptidase showed the highest activity, followed by β-1,4-glucosidase, β-1, 4-N-acetylglucosaminidase, β-D-cellobiohydrolase, α-1,4- glucosidase, arylsulphatase, and β-xylosidase (Fig. 1 and S1). In general terms, soil enzyme activity was positively linked with clay content and, to a lesser degree, with pH and SOM (Table S1; Fig. 1). L-leucine aminopeptidase was the enzyme that was most clearly driven by soil properties, while 4-N-acetylglucosaminidase followed a dissimilar pattern, being negatively related to pH and positively to SOM (Fig. 1). Except in the case of arylsulphatase, which decreased, and β-1,4-glucosidase and 4-N-acetylglucosaminidase, which remained unchanged, activities consistently increased following incubation relative to air-dried soils. However, the ranking of enzymes remained unchanged in both assays (R^2 of eight enzymes = 0.94; $P < 0.001$; Figure S1). Given that non-incubated samples were generally more responsive to the type of management, and that they represented a more direct experimental approach, we focused our description of results and discussion on non-incubated samples, but we reported the effects of management and soil type on both types of samples.

3.1. Effects of management and soil type on soil extracellular enzyme activity

The activity of C-cycling enzymes tended to be higher under organic management, but the effects slightly varied with incubation (Table 2, Fig. 2 and S2). The response of C enzymes was particularly associated with β -glucosidase, especially in sandy soils. The activity of α-glucosidase and xylosidase was generally higher in marine clay than in sandy soils (Table 2, Fig. 2 and S2). Contrary to C-enzymes, the activity of N-cycling enzymes was lower under organic management, a response that was driven by L-leucine aminopeptidase (Table 2, Fig. 2 and S2). The two N enzymes measured followed opposite patterns depending on soil type; while β-1, 4-N-acetylglucosaminidase was higher in sandy soils, L-leucine aminopeptidase was higher in marine clay soils. Phosphatase activity marginally increased in response to organic management in cereal fields, and showed a marginally significant interaction between soil type and management in incubated samples (Table 2 and S2, Fig. 2 and S2). This response was associated with an increase in sandy, but not in clay, soils. Arylsulphatase was not affected by management (Table 2, Fig. 2 and S2).

Analyses using clay as a covariate resulted in highly comparable results to the use of soil type as a categorical variable (Table S1), while restricting our analyses to farms growing cereals also yielded comparable results (Table S2), supporting the robustness of our experimental approach. Moreover, most enzymes and ratios were maintained regardless of crop type, and only LAP and AG had significantly greater activity under grass-legume mixtures (Table S3).

3.2. Effects of management and soil type on soil extracellular on enzyme activity ratios

Stoichiometric ratios of enzymes were affected by management and soil type (Table 3; Fig. 4 and S4). Carbon:N and C:P ratios were higher under organic management, particularly in sandy soils. In contrast, we found that N:P enzyme activity ratios were lower in sandy than in marine clay soils and were lower in organic than in conventionally managed soils, although this effect was only evident when restricting our analysis to cereal fields (Table S2).

3.3. Effects of management on soil extracellular enzyme activity over time

Carbon enzyme activity, the activity of the enzymes α- and β-glucosidase, xylosidase, phosphatase, and arylsulphatase, and the ratio of C:N enzymes, were affected by an interaction between management and time since conversion to organic management (Table S3; Figs. 3, 4, S3 and S4). This interaction indicated that the difference in activity of these enzymes, as well as the relative activity of C:N enzymes between organic and conventional soils, increased with time since conversion. However, this was not always driven by an increase in enzyme activity in organic soils, but in some cases was caused by a decrease in enzyme activity with time since conversion in conventional

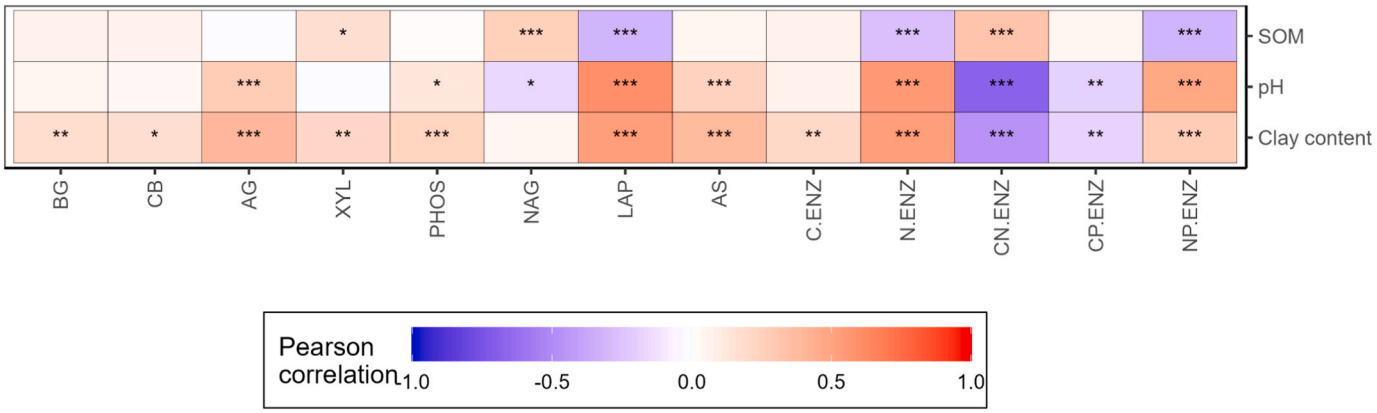

Fig. 1. Relationships between soil properties and enzyme activity and their stoichiometric ratios. BG = β-1, 4-glucosidase. CB = β-D-cellobiohydrolase. AG = α-1,4-glucosidase. XYL = β-xylosidase. PHOS = phosphomonoesterase. NAG = β-1, 4-N-acetylglucosaminidase. LAP = leucine aminopeptidase. AS = arylsulphatase. C.ENZ = C-related enzymes. N.ENZ = N-related enzymes. CN.ENZ/CP.ENZ/NP.ENZ = stoichiometric enzyme ratios.

Table 2
Effects of management, soil type, and their interactions on soil enzyme activity linked to C, N, S and P cycles, and their stoichiometric ratios. Analyses were done separately for incubated vs. non-incubated samples. Enzymes were log-transformed prior to analyses. Values in bold represent significant effects ($P < 0.05$). numDF = degrees of freedom of the numerator. denDF = degrees of freedom of the denominator. BG = β-1, 4-glucosidase. CB = β-D-cellobiohydrolase. AG = α-1,4- glucosidase. XYL = β-xylosidase. PHOS = phosphomonoesterase. NAG = β-1, 4-N-acetylglucosaminidase. LAP = leucine aminopeptidase. AS = arylsulphatase.

			Non-incubated		Incubated	
	numDF	denDF	*F*-value	*P*-value	*F*-value	*P*-value
BG, Management	1	54	4.057	**0.049**	2.440	0.124
BG, Soil	1	54	2.424	0.125	0.400	0.530
BG, Management: Soil	1	54	2.132	0.150	5.887	**0.019**
CB, Management	1	54	2.078	0.155	2.381	0.129
CB, Soil	1	54	0.906	0.346	3.693	0.060
CB, Management: Soil	1	54	0.179	0.674	0.162	0.689
AG, Management	1	54	1.960	0.167	0.657	0.421
AG, Soil	1	54	25.489	**0.000**	40.593	**0.000**
AG, Management: Soil	1	54	0.010	0.919	0.028	0.869
XYL, Management	1	54	1.295	0.260	0.877	0.353
XYL, Soil	1	54	0.384	0.538	14.485	**0.000**
XYL, Management: Soil	1	54	0.023	0.881	0.051	0.822
PHOS, Management	1	54	0.479	0.492	0.089	0.767
PHOS, Soil	1	54	11.258	**0.001**	0.099	0.755
PHOS, Management: Soil	1	54	1.833	0.181	3.176	0.080
NAG, Management	1	54	1.318	0.256	0.791	0.378
NAG, Soil	1	54	1.226	0.273	13.599	**0.001**
NAG, Management: Soil	1	54	0.318	0.575	0.066	0.799
LAP, Management	1	54	1.266	0.266	5.315	**0.025**
LAP, Soil	1	54	140.155	**0.000**	160.649	**0.000**
LAP, Management: Soil	1	54	1.377	0.246	0.017	0.898
AS, Management	1	54	0.586	0.447	0.446	0.507
AS, Soil	1	54	16.411	**0.000**	23.325	**0.000**
AS, Management: Soil	1	54	0.127	0.723	0.077	0.782
Carbon enzymes, Management	1	54	4.327	**0.042**	2.616	0.112
Carbon enzymes, Soil	1	54	2.953	0.091	0.472	0.495
Carbon enzymes, Management: Soil	1	54	1.303	0.259	3.353	0.073
Nitrogen enzymes, Management	1	54	0.570	0.454	4.224	**0.045**
Nitrogen enzymes, Soil	1	54	112.450	**0.000**	113.093	**0.000**
Nitrogen enzymes, Management: Soil	1	54	0.203	0.654	0.478	0.492
C:N ratio, Management	1	54	9.700	**0.003**	9.711	**0.003**
C:N ratio, Soil	1	54	45.532	**0.000**	44.951	**0.000**
C:N ratio, Management: Soil	1	54	3.770	0.057	6.689	**0.012**
C:P ratio, Management	1	54	4.817	**0.033**	1.965	0.167
C:P ratio, Soil	1	54	7.603	**0.008**	0.717	0.401
C:P ratio, Management: Soil	1	54	0.312	0.579	1.241	0.270
N:P ratio, Management	1	54	1.760	0.190	3.889	0.054
N:P ratio, Soil	1	54	5.263	**0.026**	37.705	**0.000**
N:P ratio, Management: Soil	1	54	2.617	0.112	1.691	0.199

fields.

4. Discussion

In this study, we tested how the activities of soil enzymes that catalyze the breakdown of organic matter responded to a transition from conventional to organic farming. We used a chronosequence of farms that transitioned from conventional to organic farming between 0 and 69 years ago, and paired each organic field with a nearby conventional field in order to account for local variation in soil and climate conditions. Using this approach, we found that organic management generally enhanced the activity of enzymes related to the C, P, and S cycles, although some of these effects were dependent on sand vs. marine clay soil (e.g., some C enzymes and phosphomonoesterase) and on time since transition (e.g., some C and N enzymes, phosphomonoesterase, and arylsulphatase). For example, some individual C-linked enzymes and phosphomonoesterase were particularly enhanced by organic farming in sandy soils.

Greater enzyme activities in organically managed soils could result from an increased use of organic compounds to fertilize the crops, the incorporation of cover crops, and the use of wider crop rotations (Tiemann et al., 2015). Under such conditions, the production of enzymes is essential to catalyze the conversion of organic compounds to mineral nutrients that can be taken up by crops (Bastida et al., 2012; Liu et al., 2017). Our results were generally consistent between air-dried and incubated samples, indicating the robustness of our results to varying conditions of sample storage. Moreover, we found that the spatial variation in soil enzyme activity was also driven by environmental factors, including texture, pH and SOM, which is in agreement with previous studies (Sinsabaugh et al., 2009, 2008). Overall, our results show how organic management may result in greater organic matter-derived bioavailability of soil nutrients (Mori et al., 2023; Stott et al., 2010) and, thus, possibly also in a more organic matter-based crop nutrition (Gunina and Kuzyakov, 2022)

4.1. Impacts of agricultural management and soil type

We found that C-cycling enzyme activities tended to be higher under organic than conventional farming, which supports our first hypothesis. These findings align with earlier work that showed that hydrolytic enzyme activities are generally higher under organic than conventional management (Mäder et al., 2002; García-Ruiz et al., 2008; Ghosh et al., 2020; Pittarello et al., 2021). The increased activity of extracellular enzymes related to C cycling under organic management may be related to higher organic inputs such as manure and compost that replace artificial fertilizers (Bowles et al., 2014). Such inputs will stimulate the activity of the soil food web and the need to produce enzymes for catalyzing the breakdown of such organic compounds (Morriën et al., 2017). For half of the C-cycling enzymes, the effect of organic farming was stronger in sandy soils than in marine clay soils, in contrast to our hypothesis.

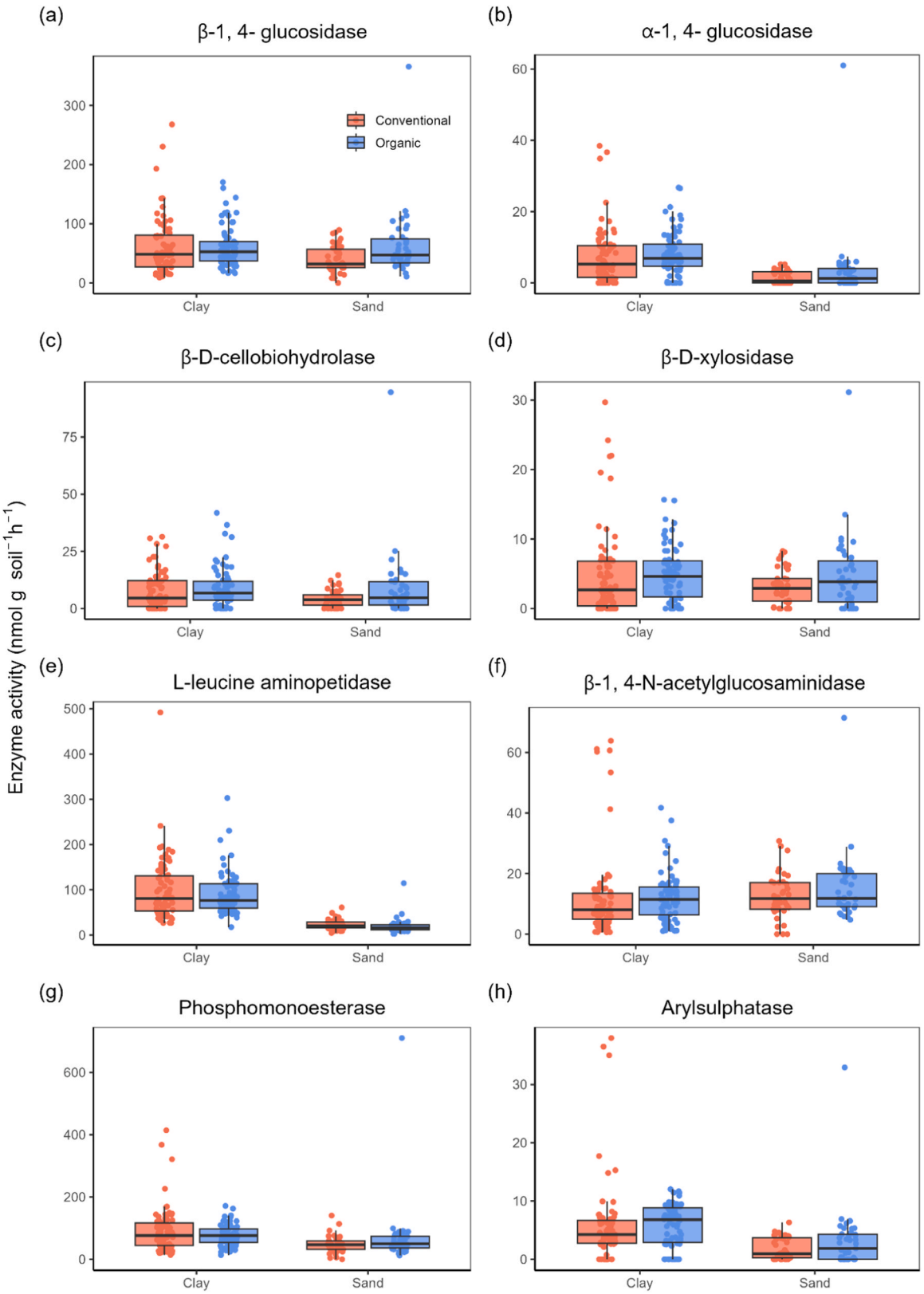

Fig. 2. Effects of management and soil type (marine clay and sand) on enzymes linked to C, N, P and S cycles. Data represent non-incubated samples. Enzymes were log-transformed prior to analyses but are represented un-transformed. For associated stats, see Table 2.

Table 3
Effects of management, soil type, time since conversion, and their interactions on soil enzyme activity linked to C, N, S and P cycles, and their stoichiometric ratios. Analyses were done separately for incubated vs. non-incubated samples. Values in bold represent significant effects ($P < 0.05$). BG = β-1, 4-glucosidase. CB = β-D-cellobiohydrolase. AG = α-1,4- glucosidase. XYL = β-xylosidase. PHOS = phosphomonoesterase. NAG = β-1, 4-N-acetylglucosaminidase. LAP = leucine aminopeptidase. AS = arylsulphatase.

			Non-incubated		Incubated	
	numDF	denDF	*F*-value	*P*-value	*F*-value	*P*-value
BG, Management	1	54	1.977	0.165	2.291	0.136
BG, Time since conversion	1	164	0.575	0.449	0.024	0.877
BG, Soil	1	54	0.948	0.335	0.349	0.557
BG, Management: Time since conversion	1	164	7.421	**0.007**	10.107	**0.002**
BG, Management: Soil	1	54	0.801	0.375	2.732	0.104
BG, Time since conversion:Soil	1	164	1.423	0.235	0.135	0.714
BG, Management: Time since conversion:Soil	1	164	0.042	0.837	1.430	0.234
CB, Management	1	54	2.837	0.098	4.059	**0.049**
CB, Time since conversion	1	164	0.330	0.566	0.002	0.961
CB, Soil	1	54	1.065	0.307	4.660	**0.035**
CB, Management: Time since conversion	1	164	3.540	0.062	3.167	0.077
CB, Management: Soil	1	54	0.361	0.550	0.273	0.603
CB, Time since conversion:Soil	1	164	1.328	0.251	0.136	0.713
CB, Management: Time since conversion:Soil	1	164	0.495	0.483	1.251	0.265
AG, Management	1	54	1.730	0.194	1.317	0.256
AG, Time since conversion	1	164	0.869	0.353	2.799	0.096
AG, Soil	1	54	17.595	**0.000**	35.106	**0.000**
AG, Management: Time since conversion	1	164	9.173	**0.003**	6.141	**0.014**
AG, Management: Soil	1	54	0.183	0.671	0.907	0.345
AG, Time since conversion:Soil	1	164	1.488	0.224	0.070	0.792
AG, Management: Time since conversion:Soil	1	164	0.049	0.825	2.132	0.146
XYL, Management	1	54	0.966	0.330	1.277	0.263
XYL, Time since conversion	1	164	0.415	0.520	0.666	0.416
XYL, Soil	1	54	0.543	0.464	18.566	**0.000**
XYL, Management: Time since conversion	1	164	8.669	**0.004**	5.740	**0.018**
XYL, Management: Soil	1	54	0.015	0.903	0.588	0.447
XYL, Time since conversion:Soil	1	164	0.717	0.398	0.003	0.956
XYL, Management: Time since conversion:Soil	1	164	0.366	0.546	1.164	0.282
PHOS, Management	1	54	0.004	0.952	0.347	0.558
PHOS, Time since conversion	1	164	3.347	0.069	4.344	**0.039**
PHOS, Soil	1	54	3.771	0.057	0.195	0.661
PHOS, Management: Time since conversion	1	164	6.012	**0.015**	8.000	**0.005**
PHOS, Management:Soil	1	54	1.318	0.256	1.293	0.260
PHOS, Time since conversion:Soil	1	164	3.276	0.072	0.925	0.338
PHOS, Management: Time since conversion:Soil	1	164	0.157	0.693	1.627	0.204
NAG, Management	1	54	0.221	0.640	0.795	0.376
NAG, Time since conversion	1	164	0.272	0.603	0.318	0.574
NAG, Soil	1	54	1.122	0.294	20.747	**0.000**
NAG, Management: Time since conversion	1	164	5.435	**0.021**	2.441	0.120
NAG, Management: Soil	1	54	0.001	0.974	0.003	0.954
NAG, Time since conversion:Soil	1	164	1.353	0.246	0.231	0.631
NAG, Management: Time since conversion:Soil	1	164	0.519	0.472	2.912	0.090
LAP, Management	1	54	0.396	0.532	6.877	**0.011**
LAP, Time since conversion	1	164	4.614	**0.033**	11.292	**0.001**
LAP, Soil	1	54	34.193	**0.000**	52.794	**0.000**
LAP, Management: Time since conversion	1	164	0.237	0.627	0.324	0.570
LAP, Management: Soil	1	54	0.099	0.754	2.285	0.136
LAP, Time since conversion:Soil	1	164	0.223	0.637	0.332	0.565
LAP, Management: Time since conversion:Soil	1	164	0.369	0.545	0.000	0.999
AS, Management	1	54	0.412	0.524	0.251	0.618
AS, Time since conversion	1	164	4.063	**0.046**	4.100	**0.045**
AS, Soil	1	54	10.669	**0.002**	16.934	**0.000**
AS, Management: Time since conversion	1	164	9.258	**0.003**	6.887	**0.010**
AS, Management: Soil	1	54	0.022	0.883	0.358	0.552
AS, Time since conversion:Soil	1	164	3.088	0.081	1.191	0.277
AS, Management: Time since conversion:Soil	1	164	0.654	0.420	1.876	0.173
Carbon enzymes, Management	1	54	2.149	0.148	2.481	0.121
Carbon enzymes, Time since conversion	1	164	0.332	0.565	0.044	0.835
Carbon enzymes, Soil	1	54	1.861	0.178	1.471	0.230
Carbon enzymes, Management: Time since conversion	1	164	7.593	**0.007**	8.244	**0.005**
Carbon enzymes, Management:Soil	1	54	0.410	0.525	0.525	0.472
Carbon enzymes, Time since conversion:Soil	1	164	1.488	0.224	0.029	0.864
Carbon enzymes, Management: Time since conversion:Soil	1	164	0.046	0.831	1.406	0.237
Nitrogen enzymes, Management	1	54	0.297	0.588	6.246	**0.016**
Nitrogen enzymes, Time since conversion	1	164	6.490	**0.012**	10.757	**0.001**
Nitrogen enzymes, Soil	1	54	31.374	**0.000**	43.546	**0.000**

(*continued on next page*)

Table 3 (*continued*)

			Non-incubated		Incubated	
Nitrogen enzymes, Management: Time since conversion	1	164	0.030	0.863	0.710	0.401
Nitrogen enzymes, Management:Soil	1	54	0.085	0.771	2.321	0.133
Nitrogen enzymes, Time since conversion:Soil	1	164	0.584	0.446	0.321	0.572
Nitrogen enzymes, Management: Time since conversion:Soil	1	164	0.093	0.761	0.077	0.782
C:N ratio, Management	1	54	9.561	**0.003**	9.429	**0.003**
C:N ratio, Time since conversion	1	163	1.254	0.264	4.251	**0.041**
C:N ratio, Soil	1	54	43.825	**0.000**	39.575	**0.000**
C:N ratio, Management: Time since conversion	1	163	4.903	**0.028**	4.202	**0.042**
C:N ratio, Management:Soil	1	54	2.068	0.156	4.491	**0.039**
C:N ratio, Time since conversion: Soil	1	163	0.051	0.821	0.586	0.445
C:N ratio, Management: Time since conversion:Soil	1	163	0.014	0.905	0.918	0.339
C:P ratio, Management	1	54	4.654	**0.035**	1.914	0.172
C:P ratio, Time since conversion	1	163	4.881	**0.029**	2.837	0.094
C:P ratio, Soil	1	54	4.949	**0.030**	1.676	0.201
C:P ratio, Management: Time since conversion	1	163	1.323	0.252	0.469	0.495
C:P ratio, Management:Soil	1	54	0.765	0.386	0.921	0.341
C:P ratio, Time since conversion:Soil	1	163	0.012	0.915	0.014	0.907
C:P ratio, Management: Time since conversion:Soil	1	163	0.001	0.972	0.615	0.434
N:P ratio, Management	1	54	1.656	0.204	3.801	0.056
N:P ratio, Time since conversion	1	163	3.215	0.075	0.091	0.764
N:P ratio, Soil	1	54	6.674	**0.013**	39.141	**0.000**
N:P ratio, Management: Time since conversion	1	163	0.420	0.518	2.569	0.111
N:P ratio, Management:Soil	1	54	2.178	0.146	0.966	0.330
N:P ratio, Time since conversion: Soil	1	163	0.297	0.587	1.089	0.298
N:P ratio, Management: Time since conversion:Soil	1	163	0.072	0.788	0.117	0.733

The stronger impacts of management on enzyme activity in sandy soils may be explained by the different organic matter content of the two types of soils (Baldrian, 2014; Sinsabaugh et al., 2008). In our study, and opposite to other studies, sandy soils had higher organic matter contents (van Rijssel et al., 2022), which may account for the greater responsiveness of enzymes in sandy soils. We also found that the effect of organic farming on phosphomonoesterase activity was particularly evident under cereal crops. Due to its high relevance for crop production, widespread limitation, and the different needs of crops, both organic and conventional farming may have managed P additions differently depending on the crop being planted, which could have obscured the overall response of phosphomonoesterase to farm management.

In contrast to our first hypothesis and previous work, the activity of N-cycling enzymes was lower under organic management, particularly in marine clay soils, as indicated by significant interactions. Although this finding opposes the general idea that organic farming enhances extracellular enzyme activity (e.g., Mäder et al., 2002; Ashraf et al., 2021), it is in line with earlier results showing lower nitrification potential under organic management despite the greater activity of enzymes linked to the C, P, and S cycles (García-Ruiz et al., 2008). This response may be attributed to the high amounts of bioavailable N often present in organic amendments, particularly in those of animal origin (e.g., NH_4^+, urea, etc.). In contrast, the high C:N ratio of plant-derived organic amendments (e.g., composts of vegetal origin), may also result in a lower need to degrade N-based compounds, and this effect can be further exacerbated in marine clay soils due to retention of the enzymes on clay particles. Nitrogen-cycling enzymes like N-acetyl-β-glucosaminidase and L-leucine aminopeptidase can also attack C-based compounds, thus implying a type of response involving several nutrient cycles that can obscure the response to management (Mori et al., 2023)

Soil extracellular enzyme activity was generally higher in incubated soil samples than in air-dried soils, but this did not generally alter the impact of management or soil type on soil extracellular enzyme activity, with slight differences. In air-dried soils, enzyme activity may be lower due to sorption of enzymes as micropores dry up and force enzymes in the soil solution back onto the mineral surface (Quiquampoix et al., 1993; Ranjan and Sonalika, 2022). Activities in air-dried soils may thus better reflect mineral-associated or stabilized enzymes in the sample (Margenot et al., 2018; Wade et al., 2020). In addition, under incubation the soil microbial community is reactivated, and they may start to produce enzymes again, resulting in higher overall activity. We have unpublished evidence that extracellular enzyme activity in air-dried soils did not recover up to field levels after rewetting, thus implying that, regardless of incubation, our results could be an underestimation of enzyme activities in freshly collected soils.

4.2. *Enzyme stoichiometry*

Our second hypothesis assumed that, under organic farming, enzymes related to the acquisition of C compounds would increase more than enzymes related to the acquisition of N and P (Sinsabaugh et al., 2009, 2008). Our results generally supported our hypothesis, as C:N and C:P ratios were higher under organic management, particularly in sandy soils. However, enzymes can originate not only from living organisms but also from dead microbes (necromass), plant roots, plant residues, and soil animals. Hence, alterations in the ratios of soil enzymes can be interpreted as a reflection of a shifting ecosystem-level metabolism, and not only as a plant or microbial response. Moreover, C-based compounds are not only broken down by C-related enzymes but also by N- and P-related enzymes (Mori et al., 2023), which means that we should be cautious when evaluating the response of stoichiometric ratios of enzymes. For example, in some cases, C limitation can lead to the production of phosphatases (Spohn and Kuzyakov, 2013; Wang et al., 2016) or aminopeptidases (Norman et al., 2020). Caution should also be taken when linking rations of soil extracellular enzymes to microbial resource use because enzymes can persist in the soil for long periods of time following secretion and, therefore, may not necessarily reflect current microbial demand or biochemical processes (Burns, 1982).

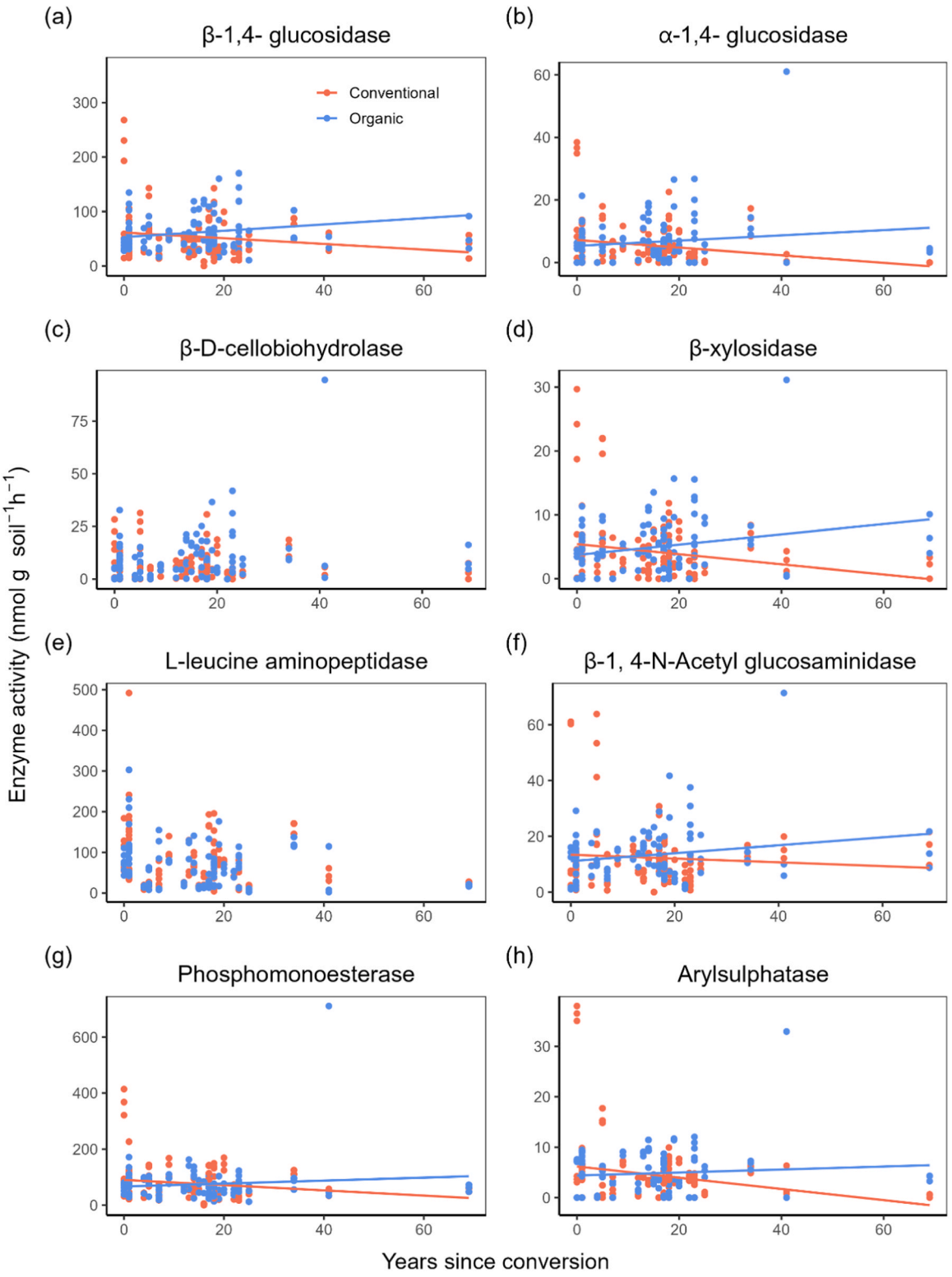

Fig. 3. Soil extracellular enzyme activity under conventional and organic management over time (0–69 years since conversion) for the individual soil enzymes linked to C, N, S and P cycles. Red = conventional; Blue = organic. Lines represent significant interaction effects. Data represent non-incubated samples. For associated stats, see Table 3.

4.3. Impacts of time since conversion to organic management

We found that the differences for α-glucosidase, β-glucosidase, xylosidase, phosphomonoesterase, arylsulphatase, and the ratio of C:N enzymes between organic and conventional farming increased with time since conversion to organic farming, as indicated by significant management by time interactions. Additionally, the activity of all C-related enzymes also increased with time since conversion in organic fields. These findings support our third hypothesis, which stated that impacts of organic farming would amplify over time. Divergence in activity driven by enhanced activity in organic fields may be caused by impacts of agricultural management on the soil microbiome becoming

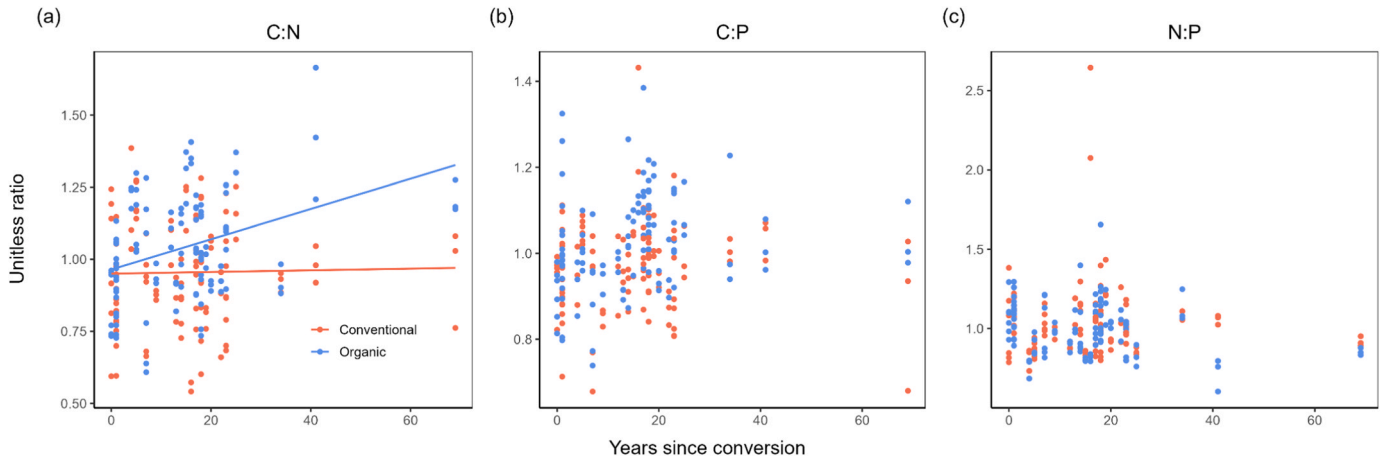

Fig. 4. Ratios of soil extracellular enzyme activity under conventional and organic management over time (0–69 years since conversion). Red = conventional; Blue = organic. Lines represent significant interaction effects. Data represent non-incubated samples. For associated stats, see Table 3.

more apparent a few years after conversion due to legacy effects that prevented a quick transition (Hartmann and Six, 2023). This shifting microbial community may influence the release of certain enzymes.

Although we found divergence over time for the activity of some enzymes, this was not always driven by increases in enzyme activity in organic fields. For some enzymes such as α-glucosidase and arylsulfatase, we found that enzyme activity was reduced in conventional fields with time since conversion of the organic neighbor. This finding was surprising as we expected neutral responses to time in the conventional fields, because conventional fields were never converted, and they were only plotted along the same time axis as the organic fields as controls. This finding suggests therefore that our time axis may partly be confounded with other variables that were unaccounted for. None of the measured abiotic soil properties could explain the apparent time gradient in conventional fields, but biotic variables, such as fungal community composition and diversity, also changed with time since conversion in conventional fields (van Rijssel et al., 2022). This suggests at least that changes in the microbiome may have driven changes in the enzyme activity in our fields. For example, the shifts in fungal diversity over time could underlie the changes in xylosidase with time, as fungi are the main producers of this enzyme (Baldrian, 2014).

Although a chronosequence approach is a valuable method to evaluate long-term impacts of changes in management, there were also some other limitations to our experimental approach. First, since the 1950s, inputs to agriculturally managed soils (e.g., chemical fertilizers, biocides, animal manure) have increased (Erisman et al., 2008). As a result, the oldest organic fields in our study have never been exposed to such practices and, therefore, may not represent the trajectory that more recently converted organic fields underwent. Still the trends with time did not seem to level off towards the older fields, indicating that the full impact of agricultural transitions on soil functions may take decades (Durrer et al., 2021; Liang et al., 2014). The fact that impacts of land use change on enzyme activities takes time is also in agreement with findings following the abandonment of agricultural land and restoration of natural vegetation (Raiesi and Salek-Gilani, 2018; Zhang et al., 2015). In addition, our approach reveals that spatial variation of underlying variables, such as variation in soil properties, practices applied by individual farmers, and the type of crop sampled, may obscure the impact of time since conversion on enzyme activity and other ecosystem properties. Moreover, the fact that our sampling took place between June and July may have also obscured some of the responses, emphasizing the importance of using proper controls in a chronosequence approach to be able to dissect the impact of time since conversion on soil functioning more precisely.

5. Conclusions

We demonstrated that conversion from conventional to organic farming enhances the activity of soil enzymes, particularly on sandy soils, and that for some of these enzymes, i.e., α-glucosidase, β-glucosidase, xylosidase, phosphomonoesterase, and arylsulfatase, differences in enzyme activity between organic and conventional fields increased with time. Enhanced enzyme activity in organically managed soils may suggest a greater potential for crop residue decomposition and higher rates of nutrient cycling. We thus speculate that, in such systems, soils may be able to provide more bioavailable nutrients to microbes and plants and thus support greater plant growth and a more active soil food web with lesser inputs of mineral nutrients in the form of synthetic fertilizers. Finally, our study suggests that shifts in soil enzyme activity upon land use conversion may take time and, therefore, it may be critical to apply management measures that speed up the transition towards a more nature-based, organic agriculture that contributes to safeguarding the biodiversity and functioning of agricultural soils.

CRediT authorship contribution statement

G.F. (Ciska) Veen: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Wim H. van der Putten:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Lilia Serrano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Guusje J. Koorneef:** Writing – review & editing, Methodology, Data curation. **Sophie Q. van Rijssel:** Writing – review & editing, Methodology, Data curation. **Andrew J. Margenot:** Writing – review & editing. **Raúl Ochoa-Hueso:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

[Data set]. Zenodo.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109202.

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