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Original Research

Hydrolases Related to C and N Cycles and Soil Fertility Amendment: Responses to Different Management Styles of Agro-Ecosystems

Ligita Baležentienė*

Aleksandras Stulginskis University, Studentų 11, Akademija, Kaunas distr., Lithuania

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Abstract

Extracellular enzyme activity is proposed as a universe index of soil fertility and contamination. To ascertain and to make a comparison of bioactivity variation during vegetation period, a variety of soil samples (including Hapli-Epihypogleyic Luvisol, Albi-Epihypogleyic Luvisol, and Hapli Albic Luvisol) were collected from rotation fields of different fertilizing and farming systems, namely extensive (ExF), conventional (CF) and organic (OF). The objective of this study was to determine soil bioactivity responses (saccharase and urease activity) on different land management systems and the main recourses of ecological factors such as soil genesis type, content of soil organic carbon (SOC), total nitrogen (N), and C/N ratio. The trial was comprised of eight experimental plots (three land management systems and two or three crop groups: legumes, gramineous, rapeseed, and bare fallow).

The lowest mean of urease (1.13 mg NH_4^+-N g^{-1}) and saccharase (8.40 mg CG $g^{-1}\cdot 24\ h^{-1}$) activity was observed in abandoned grassland soil where mineral fertilizers were not applied. A general increase of hydrolytic enzyme activity has been observed in soil under conventional (144-413% of urease and 49-50% of saccharase) and organic (219-269% of urease and 78-221% of saccharase) management as compared with abandoned grassland. A strong correlation between enzyme activity and SOC (r = 0.7) was determined. Therefore, it can be concluded that farming management and soil fertility might be responsible for the different levels of enzyme activities in soil.

Keywords: enzyme activity, farming management, crops

Introduction

Fertility is the most practically important soil characteristic, which depends on soil biochemical components and their ratio. About 3/4 of the organic carbon contained in terrestrial ecosystems and the majority of organic nitrogen are found in plant residues and soil organic matter [1]. In addition, soil biota influences materials and energy metabolism substantially, thus determining soil fertility. Soil fertility plays an important role in the sustainable development of terrestrial agro ecosystems and mainly depends on results of organic matter decomposition to low molecular weight and dissolved organic compounds [2, 3]. However, recent concern regarding long-term productivity and sustainability of agro-ecosystems has resulted in the application of various bio-indices and biological methods. These methods have led to the development and protection of soil resources as well as fertility. Soil biota responses to environmental changes is integrating the standpoint of vitality of soil fertility [4].

*e-mail: ligita.balezentiene@asu.lt

Biota governs the soil role in metabolism processes of materials and energy in ecosystems and represents an integrated index of soil physical-chemical conditions and soil quality. Moreover, soil biodiversity confers stability over stress and disturbance, but the mechanism is not yet fully understood. Thus, biological equilibrium reached might be different from the original populations [5]. Extracellular enzymes are produced by plants, animals, and microorganisms. These may be present in dead cells or cell debris and also adsorbed by clay or incorporated into humic substances [4]. Dissolved organic matter is produced by soil enzyme-catalyzed depolymerization of organic matter and is comprised of low molecular weight compounds that are often water-soluble and thus more accessible to biota assimilation as energy, carbon, and nutrient sources. Thus, soil microorganisms and enzymes are the primary mediators of soil biological processes, including organic matter degradation, mineralization, and nutrient recycling [6, 7]. Hydrolytic enzymes make nutrients available for plants and soil microorganisms from a wide range of complex substrates and are influenced by a wide range of soil peculiarities such as pH, organic matter, and texture, and also by farming management and anthropogenic impacts [6]. Hydrolytic enzymes, namely saccharase and urease, are related to the C and N cycles, which are the fundamental factors in forming soil fertility [4]. Among other indices, enzyme activity is proposed as an index of soil fertility [6] or contamination [8]. These enzymes are non cellular and persist in the soil matrix, even though they are sensible for abiotic factors [29], especially for fertilizers. Some references have pointed out an increase in abundance of microorganisms as well as in some enzyme activity in the case of applying organic and mineral fertilizers [6, 7, 25]. Past studies have shown that high doses (>120 kg·ha^{-1}) of mineral fertilizers as well as the species of cultivated crop will change microorganisms and ferment composition and abundance [9]. Therefore, evaluation of enzyme activity is an actual bio-indicator specific to soil organic matter biochemical alteration [10].

In the mid 20th century traditional and diverse management of farming was replaced by modern, highly specialized conventional agriculture. Today nearly 40% of the European Union (EU) area is agricultural land and thus mainly used for production of food and raw materials [11]. Intensive agriculture was achieved by application of high cropping technologies based on high-yielding cultivars and hard rates of agro chemicals (synthetic fertilizers and pesticides). These enumerated agricultural measures determined tremendous increases in food production. In consequence, the intensive management of agriculture negatively affected both abiotic (soil, water, air, habitats) and biotic (species, communities, and biodiversity) environmental resources [12]. Therefore, recent developments in EU policies to foster environmentally friendly farming practices and agricultural surplus reduction have led to widespread interest in organic farming [13]. Hence, over the last decades, organic management has been introduced in order to preserve soil sustainability by allowing the maintenance and even the increase of soil fertility through the use of farmyard manure, the omission of synthetic fertilizers and pesticides, and the lower use of high energy-consuming foodstuffs. Following the implementation of EU regulation 2092/91 [14], organic farming has been developing in Lithuania during the last two decades, and now occupies over 0.13 M ha. The demand for suitable soil quality indicators, evaluation, and monitoring of the impact, and measurements of the success of any specific agricultural practices, has increased due to the implementation of environmentally friendly agricultural policies [14].

The main aim of this study was to evaluate the impact of farming management systems (conventional, organic, and extensive) and different crops (legumes and gramineous) on activity of urease and saccharase related to the C and N cycles in a 15-year period on different soils. In addition, this study addressed the question of which main biochemical indicators are suitable for soil fertility rating.

Materials and Methods

Soil samples were collected in accordance with ISO 10381-2:2002 from different farming fields (Aleksandras Stulginskis University) from: extensive (abandoned barley field ExF b-cg, grassland ExF g and fodder galega (*Galega orientalis* Lam.) stand ExF lga), conventional (winter wheat w for grain, legumes mixtures ov for fodder), and organic (winter wheat w for grain and legumes mixtures ov for fodder) rotation crops in the middle of vegetation, June 2007-09. The organic farm superseded the conventional farm in 1996 and has since been active in the Training Farm. Phytocoenoses of extensive farming consists of an abandoned barley field, (>20 year), perennial legume fodder galega, and abandoned grassland (Table 1).

Soil samples were taken from agricultural fields of 35 m × 35 m size at 8 sites (GPS coordinates presented in Table 1). A cylindrical auger (2 cm in diameter) was used for soil sampling, done randomly from nine different locations of the same field. Samples were then mixed thoroughly to prepare a composite mixture. In each field plot two separate soil samples were collected from surface horizon (0-25 cm depth) and dried at ambient temperature. Fractions of soil samples were ground and sieved through a 2 mm mesh sieve. Soil types were classified in accordance with FAO/UNESCO [15].

This study relies on previous chemical analysis data obtained by performing a joint project presented by Sabiene [16]. Content of organic C and total nitrogen were determined as the main substrates for assayed enzymes. SOC was determined through dry combustion method (ISO 10694:1995) and N was measured as Kjeldahl nitrogen (ISO 10694). In addition, C/N ratio was calculated (Table 1). All treatments and measurements were replicated two times.

Soil bioactivity was characterized by applying bioassay of hydrolytic enzyme activity. The enzymatic activity was determined in air-dried soil samples. Saccharase (invertase) (EC 3.2.1.26) activity was measured according to the modified [17] Hofmann and Seegerer method. 100 mg of soil

Table 1. Trial design during 2007-09.

Management type	Crop rotation	Treatment acronym	Fertilizing	Year	Soil classification	Location
Conventional farming Extensive farming (abandoned crop)	barley, couch-grass, couch-grass	CF b ExF b-cg	$N_{120}P_{50}K_{60}$ 0 0	2007 2008 2009	Hapli-Epihypogleyic Luvisol (LVg-p-w-ha)	54º52'40"N 23º50'99"E
Extensive farming (abandoned grassland)	grassland	ExF g	0 0 0	2007 2008 2009	Hapli-Albic Luvisol (LVe-ha)	54º53'83"N 23º51'61"E
Extensive farming (abandoned crop)	legumes (galega)	ExF lga	0 0 0	2007 2008 2009	Hapli-Albic Luvisol (LVe-ha)	54º53'32"N 23º40'85"E
Conventional farming (synthetic fertilizers, weeds controlled by tillage and herbicide)	oat-vetch, winter wheat, barley-clover	CF ov-w-bcl	$N_{60}P_{50}K_{60}$ $N_{120}P_{50}K_{60}$ $N_{60}P_{50}K_{60}$	2007 2008 2009	Hapli-Epihypogleyic Luvisol (LVg-p-w-ha)	54º52'32"N 23º51'48"E
Conventional farming (synthetic fertilizers, weeds controlled by tillage and herbicide)	oat-vetch, barley	CF ov-b	$N_{120}P_{50}K_{60}$ $N_{120}P_{50}K_{60}$	2008 2009	Albi-Epihypogleyic Luvisol (LVg-p-w-ab)	54º51'66"N 23º48'40"E
Conventional farming (synthetic fertilizers, weeds controlled by tillage and herbicide)	winter wheat, rapeseed, bare fallow	CF w-r-bf	$N_{120}P_{50}K_{60}$ 0 0	2007 2008 2009	Albi-Epihypogleyic Luvisol (LVg-p-w-ab)	54º52'92"N 23º51'42"E
Organic farming (*certificated 15 yrs)	oat-pea, barley, barley-clover	OF op-b-bc	Manure, 80 $t \cdot ha^{-1}$	2007 2008 2009	Hapli-Epihypogleyic Luvisol (LVg-p-w-ha)	54º52'44"N 23º51'39"E
Organic farming	winter wheat, oat-pea, barley	OF w-op-b	Manure, 80 $t \cdot ha^{-1}$	2007 2008 2009	Hapli-Epihypogleyic Luvisol (LVg-p-w-ha)	54º52'30"N 23º51'40"E

*Organic certification by the EKOAGROS (Lithuanian Committee for Organic Agriculture)

was incubated at 37ºC for 2 h, with 400 ml of a citrate phosphate buffer at pH 4 and 400 μl 10 M of substrates. After incubation, the samples were centrifuged at 14,000 x g for 3 min and 200 ml of the supernatant were used to determine conventional glucose (CG, $mg \cdot g^{-1}$ of air dried soil) released. Three ml of 2% Na_2CO_3 solution (w/v) was added to the supernatant to stop the reaction, and absorbance measured at 410 nm. Controls were used simultaneously for each sample, but substrate was added to controls after incubation. Urease (EC 3.5.1.5) activity was assayed by employing the modified [17] Hofmann and Schmidt spectrometric method. When determining urease activity, soil (5 g) was incubated for 24 h at 37ºC with 1 ml of 0.08 M urea solution. In the controls, 1 ml of deionized water was used instead of the urea solution. For the preparation of standards, 0, 0.25, 0.5, 1, 2.5, 4, 6, 8, and 10 ml of a 100-fold diluted 71.4 mM ammonium chloride solution were made up to 10 ml using 2 M KCl and then the NH_4^+ concentration was determined in aliquots of 0.5 ml as reported above. Urease activity was expressed in mg NH_4^+ -$N \cdot g^{-1} \cdot 24\ h^{-1}$ of air-dried soil.

Variation of annual temperatures and precipitations were observed during the study period 2007-09. Average temperatures ranged between -6.2ºC (2007) and 18.5ºC (2005); average precipitation ranged between 8.6 mm (2009) and 148.7 mm (2007).

The confidence limits of the data were based on one-way analysis of variance by *Anova* (in case of significant interactions) followed by *post hoc* Turkey theoretical criterion. The least significant differences between treatment means were determined using Fisher's least significant differences (LSD_{05}). LSD, standard error (SE), correlation coefficient (r) has been calculated at the level of statistical significance $p<0.05$. Results of urease and saccharase activity are presented as a mean of 4 independent analyses at the 0.05 probability level.

Results and Discussion

As extracellular enzymatic activity followed their substrate availability within soil profile, hydrolytic enzymes (saccharase and urease) were undertaken as indicators indirectly identifying responses to different crops (gramineous, and legumes) and farming types (extensive/abandoned, organic and conventional) in this study. Those essential for humus turnover and nutrient release or immobilization enzymes evolved into C and N cycles of essential life and nutritious elements mostly depend on SOC [6]. We investigated the 15-year effects of organic management on soil quality resembled by chemical and biological parameters of the soil. Responding to references [7, 18], farming management type and crop rotation indicated significant impact on soil fertility indices, namely on SOC (r = 0.7), N (r = 0.4), and C/N (r = 0.7) (Fig. 1). Eventually we identified hydrolytic enzyme (saccharase and urease) fluctuation in the same background.

The most prevalent and investigated enzyme in nature, saccharase, is produced by plants and microbes catalyzing saccharose (raffinose, stachiose, etc.) hydrolysis to glucose and fructose characterizing changes of SOC in soil. As referred to by Singh and Kumar [19], saccharase is used as a reliable index of soil bioactivity and fertility due to association with humus, K_2O and P_2O_5 content – the indices for which relate to the farming system. Therefore, saccharase activity, (with the exception for CF crop stands) was observed to be significantly different and dependent on farming type as well as crops during 3 study years (r = 0.3; Fig. 2). Three years of conventional farming significantly induced the highest saccharase activity in winter wheat stand (27.00 mg CG g^{-1}), supposedly due to appropriate aeration conditions [28] and sufficient C containing substrate supply [6]. The lower saccharase activity was

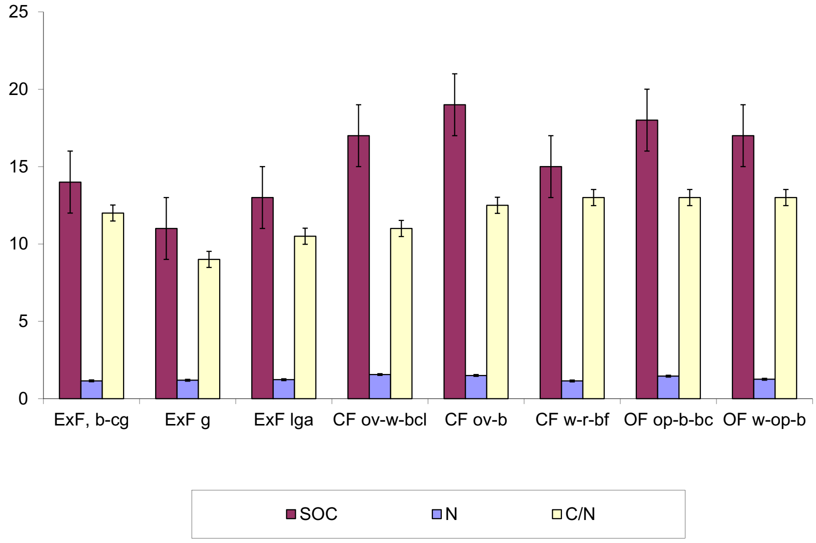

Fig. 1. Soil carbon, nitrogen, and their ratio in different field management (mean±SE, p=0.05). Mean values of 3 years. Extensive farming (abandoned crop) barley; couch-grass – ExF b-cg; extensive farming (abandoned grassland) grassland – ExF g; extensive farming (abandoned crop) legumes (galega) – ExF lga; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide) oat-vetch; winter wheat; barley-clover – CF ov-w-bcl; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide) oat-vetch; barley – CF ov-b; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide); winter wheat; rapeseed; bare fallow-CF w-r-bf; organic farming (certificated 15 yrs) oat-pea; barley; barley-clover – OF op-b-bc; organic farming winter wheat; oat-pea; barley-OF w-op-b.

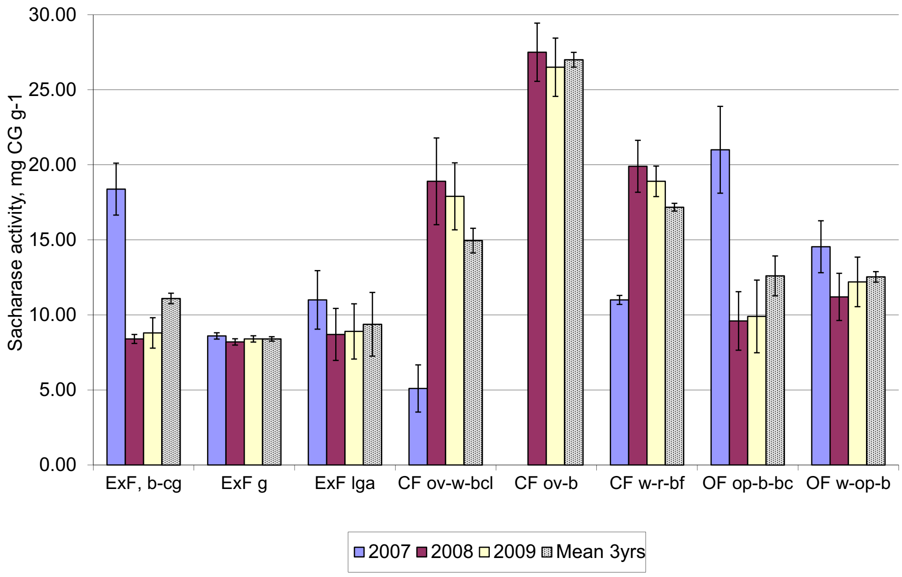

Fig. 2. Saccharase activity responses to farming type and different crop stands (mean±SE, $p < 0.05$). Extensive farming (abandoned crop) barley; couch-grass – ExF b-cg; extensive farming (abandoned grassland) grassland – ExF g; extensive farming (abandoned crop) legumes (galega) – ExF lga; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide) oat-vetch; winter wheat; barley-clover – CF ov-w-bcl; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide) oat-vetch; barley – CF ov-b; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide); winter wheat; rapeseed; bare fallow-CF w-r-bf; organic farming (certificated 15 yrs) oat-pea; barley; barley-clover – OF op-b-bc; organic farming winter wheat; oat-pea; barley-OF w-op-b.

observed in CF fallow (17.18 mg CG g^{-1}) and CF mixtures with legumes (15.87 and 14.95 mg CG g^{-1}, respectively), as compared with that of CF w. wheat treatment (Fig. 2).

Relatively high saccharase activity in stands with legumes could be explained by the formation of nodules on leguminous plant roots. As Cooper [20] referenced, nodulated plant root produced glycosides containing flavonoids such as C substrate, which is decomposed by saccharase.

Extensive farming did not stimulate indices of soil fertility (SOC, N accumulation, and C/N rise) and saccharase activity, which significantly observed the lowest during the three-experiment year in cereal (8.40 mg CG g^{-1}) and legume (9.38 mg CG g-1) crops as compared with CF, OF, and ExF [9, 21]. Response of saccharase activity to different farming types could be explained by its strong correlation with SOC content (r=0.7), which varied across different farming systems and ranged between 4.86 in ExF meadow and 12.4% in IF w. wheat stands. The highest mean values of soil fertility indices were observed in fields of conventional (17 g·kg^{-1} SOC, 1.4 g·kg^{-1} N, and 12 C/N ratio) and organic (17.5 g·kg^{-1} SOC, 1.36 g·kg^{-1} N, and 13 C/N ratio) management system. In accordance with chemical parameters of soil fertility, the highest mean activity of saccharase was observed in CF (19.71 mg CG g^{-1}) and OF (12.57 mg CG g^{-1}), respectively. Therefore, our study demonstrated that conventionally and organically managed soils exhibited greater saccharase activity due to beneficial conditions for accumulation of SOC (r = 0.7; Fig. 3) and

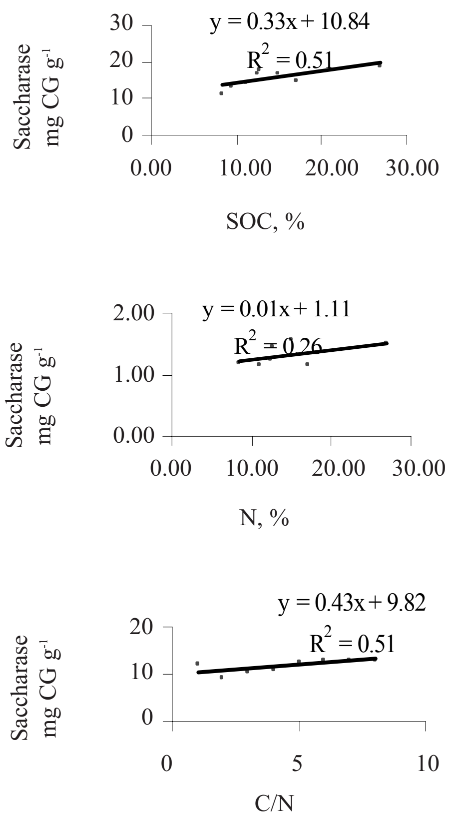

Fig. 3. Correlation between saccharase activity and soil fertility indices ($p<0.05$).

forming a favourable C/N ratio (r = 0.7, Fig. 1), which, in turn, guarantees a sufficient amount of C-containing substrate [22].

As reported in numerous studies [23, 24], SOC content guaranteed decomposed polysaccharides mass – a proper substrate (saccharase) promoting saccharase activity. Therefore, it could be concluded that most active SOC decomposition was observed in CF and OF, where the highest SOC content was determined. Uncultivated and unfertilized soils of ExF did not accumulate organic matter and other elements important when forming soil fertility. Therefore, the lowest SOC content (5.67-4.86 g·kg^{-1}) and saccharase activity (8.6-11 mg CG g^{-1}) were observed.

Urease activity represented potential ammonification rate and has attracted considerable attention due to increasing application of urea as fertilizer to improve soil fertility [25, 26]). Similarly, as in the case with saccharase, the different soil urease activity was observed for different farming and crop types (Fig. 4). Urease activity was related to nitrogen-containing substrate dynamics in response to land management intensity and crops (r=0.6). The highest urease mean activity was observed in CF and OF wheat stands (5.79 and 4.16 mg), possibly due to applied heavy nitrogen rates ($N_{100-120}$). This trend corresponded with references [22] to significantly higher potential ammonification in the conventional farms consistent with the long-term application of ammonium or urea as the main fertilizer source, and suggests the presence of an important community of autotrophic nitrifying bacteria.

Higher urease activity indicates that plants continue to assimilate nitrogen inputs [19]. These high annual inputs are targeted to provide heavy harvest in commodity-based crops. Nonetheless, the rise of urease activity correlated (Fig. 5) stronger with SOC (r = 0.7) or C/N ratio (r = 0.7) than that with total nitrogen content (r = 0.6). Thus urease is related to total soil fertility [24]. A decrease of urease activity was observed in soil of EF abandoned grassland (1.13 mg NH_4^+-N g^{-1} 24 h^{-1}) and barley-coach grass (1.67 mg NH_4^+-N g^{-1} 24 h^{-1}) due to the lowest total N (1.2 g·kg^{-1}) and SOC (11 g·kg^{-1}) content. Due to lower rates of soil quality indices (12.7 g·kg^{-1} SOC, 1.19 g·kg^{-1} N, and 10.5 C/N ratio), the lowest urease mean activity (2.27 NH_4^+-N g^{-1} 24 h^{-1}) was observed in fields of extensive farming. Increases of soil fertility indices in conventional (17 g·kg^{-1} SOC, 1.40 g·kg^{-1} N, and 12.7 C/N ratio) and organic (17.5 g·kg^{-1} SOC, 1.36 g·kg^{-1} N, and 13 C/N ratio) farming indicated better agronomic management there. Land management stimulated the increase of mean urease activity in conventional (3.93 mg NH_4^+-N g^{-1} 24 h^{-1}) and organic (3.88 mg NH_4^+-N g^{-1} 24 h^{-1}) farming.

In summary, soil chemical indices (N and SOC) and assessed hydrolase activity indicated positive impact and successful agricultural practices of conventional farming in studied sites and correspond with Zibilske and Bradford [26]. Nonetheless, C/N ratio (13.0) was significantly higher and more crop-favorable in organic farming than in conventional (12.2). Agro chemical properties alone were not sensitive enough to track relatively subtle soil quality improve-

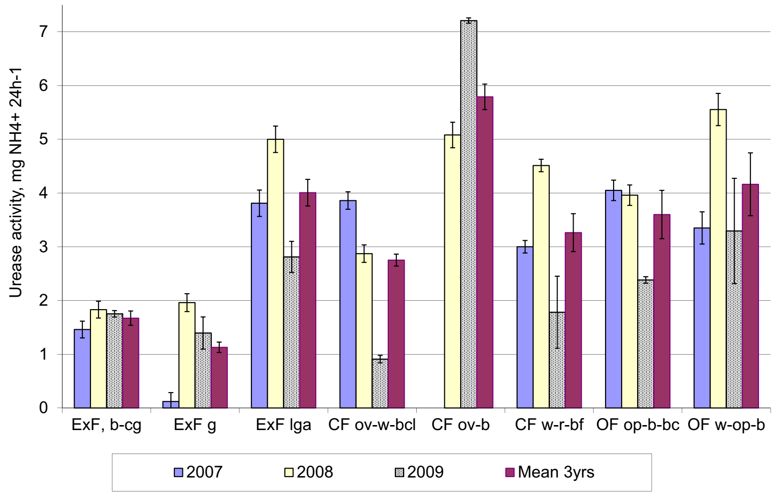

Fig. 4. Potential ammonification rate responses (r = 0.6) to farming type and different crop stands (mean±SE, p<0.05). Extensive farming (abandoned crop) barley; couch-grass – ExF b-cg; extensive farming (abandoned grassland) grassland – ExF g; extensive farming (abandoned crop) legumes (galega) – ExF lga; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide) oat-vetch; winter wheat; barley-clover – CF ov-w-bcl; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide) oat-vetch; barley – CF ov-b; conventional farming (synthetic fertilizers; weeds controlled by tillage and herbicide); winter wheat; rapeseed; bare fallow-CF w-r-bf; organic farming (certificated 15 yrs) oat-pea; barley; barley-clover – OF op-b-bc; organic farming winter wheat; oat-pea; barley-OF w-op-b.

ments in farms over the past 15 years since the shift from conventional to organic farming in the investigated sites. As distinct from some references [21, 27], our results using hydrolytic enzymes suggest that soil functionality (i.e. its capacity to cleave organic compounds) was also enhanced under conventional management. Indeed, 15 years of practices undertaken routinely were not sufficient for improving soil quality indices in the organic farms covered in this study. This emphasizes that the differences between organic and conventional farming are more gradual and that other sources of variation to account for are present. Observed variation of the assayed soil hydrolases activity might have occurred due to different site properties (i.e. soil type, landscape characteristics, or environmental conditions) in accordance with van Diepeningen et al. [18]. This finding suggested that a high number of plots showing a wide range of soil properties and different management history should be investigated in order to employ soil enzyme activities as an assessment tool to estimate beneficial environmental effects of friendly agricultural management practices.

Conclusion

The assessment of soil enzymes can be used to indicate biological activities and natural biochemical processes in soil. Soil enzyme activities have the potential to provide a uniquely integrated biological and biochemical assessment of soils because of their relationship to soil biota, easiness of measurement, and rapid response to all sorts of changes, i.e. anthropogenic, agronomic, chemical, and environment conditions. In our experiment, conventional and organical faming promoted the highest enzyme activity. Enzyme activity correlated stronger with SOC (r = 0.7) or ratio C/N (r = 0.6) than that with total nitrogen content (r = 0.5-0.6). Significantly higher mean value of saccharase (27.00 and 12.6 mg CG g^{-1}) and urease (5.78 and 4.16 mg NH_4^+-N g^{-1} 24 h^{-1}) were observed in CF ov-b and OF w-op-b rotation fields respectively. In most of the studies, it was observed that enzymes behave differently with different SOC content. Fluctuation in enzyme activities in experimental soil

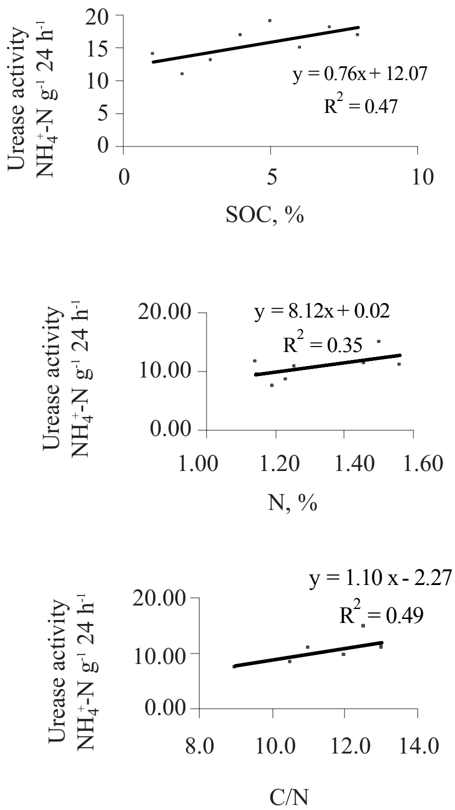

Fig. 5. Correlation between urease activity and soil fertility indices (p < 0.05).

might have occurred due to different agronomic practices and soil fertility conditions during the crop seasons. More intensive studies are required to evaluate the effects of different farming practices on important soil enzymes concerning soil health and fertility. Statistically significant differences between observed farming management types and soil biochemical indices have revealed the relevance of enzyme activity as a proxy indicator for evaluating soil fertility.

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