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Biodynamic compost effects on soil parameters in a 27-year long-term field experiment

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Abstract

Background: Soil samples were taken after 27 years from a long-term field experiment to study the effects of composted pure cattle farmyard manure (FYM) and two FYM treatments with biodynamic preparations on soil chemical and microbiological properties.

Methods: Soil organic carbon (SOC), total nitrogen, basal respiration, fungal ergosterol, microbial biomass C (MBC) and nitrogen (MBN) were analyzed in a 6-field crop rotation system, conducted as a randomized block design with six replicates. The multi-substrate-induced respiration (multi-SIR) approach was used to assess microbial functional diversity by the respiratory response of 17 low molecular weight organic substances.

Results: All composted FYM treatments revealed generally positive effects on SOC, total N, basal respiration, MBC, and MBN in contrast to control without FYM. Only fungal ergosterol was not increased by FYM application. After 27 experimental years, discriminant function analysis of multi-SIR data not only revealed significant general effects of biodynamic preparations, but was also able to differentiate between the sole application of the *Achillea millefolium* preparation and the standard application of all 6 biodynamic compost preparations.

Conclusions: The *Achillea* preparation was specifically able to improve the N status of the microbial community as indicated by the higher catabolic use of D-glucosamine as well as the amino acids γ-aminobutyric acid, L-cysteine, and L-leucine. The reason for different effects of the sole *Achillea* preparation and all 6 preparations cannot be explained by the current study.

Keywords: Farmyard manure, biodynamic agriculture, *Achillea* preparation, Multi-substrate-induced respiration, Microbial biomass

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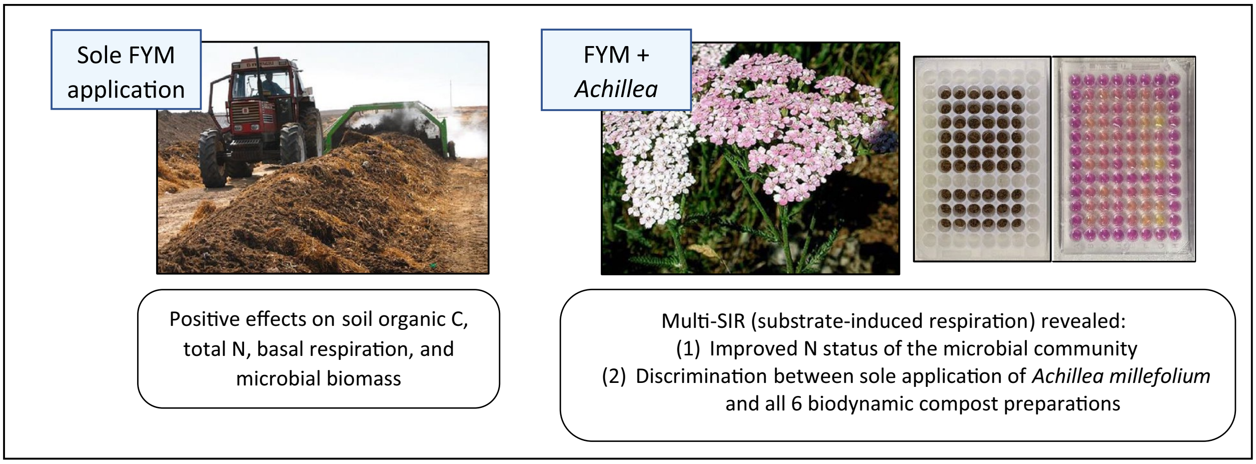
Graphical Abstract

Composted farmyard-manure and biodynamic preparations effects on soil chemical and microbial properties in a 27-year long-term field experiment



Background

Farmyard manure (FYM) is one of the main tools used in organic agriculture to improve soil fertility [1] and to increase soil organic carbon (SOC) storage [2–5], which promotes climate change mitigation [6, 7], erosion landslide reduction and livelihood benefits [8], while environmental quality (soil, water, and air) is enhanced [9]. This contrasts with the inorganic fertilization of conventional agriculture, where typically annual crops are featured, promoting their growth with synthetic fertilizers [10] and chemical pesticides [11], disturbing soil through tillage [12] and leaving the soil bare in no crop-growing periods. Thus, FYM application is an important alternative agricultural practice that also reduces other environmental burdens, such as biodiversity damage [13, 14]. Biodynamic agriculture is the oldest certified organic farming method and has looked for alternative approaches since inorganic nitrogen fertilizers were first applied [15, 16]. Today, over 251,000 ha have been certified according to Demeter standards in 55 countries [17].

In biodynamic agriculture, FYM is composted and usually supplied with six preparations as a standard application. The compost biodynamic preparations consider yarrow (*Achillea millefolium* L.) blossoms, chamomile (*Matricaria recutita* L.) blossoms, stinging nettle (*Urtica dioica* L.) shoots, oak (*Quercus robur* L.) bark, dandelion (*Taraxacum officinale* L.) flowers, and valerian (*Valeriana officinalis* L.) flower extract, numbered 502 through 507 [18, 19]. Previous reports often showed no clear differences between traditional and biodynamically prepared compost [20, 21] or biodynamic practices [22]. However, numerous peer-reviewed research papers published over the past decade [23–32] point to the possibility of positive biodynamic farming effects in controlled long-term field experiments, case studies, and on-farm research trials. These positive influences comprise effects on soil quality [31, 33], soil biodiversity [13, 23], N and P availability to soil microorganisms, as well as balancing contrary effects of microbial functional diversity [28]. Despite these positive observations, there is ongoing debate about how biodynamic preparations function [15]. Due to the increasing focus on global biodynamic production over the past few years [17], this management approach is clearly worth studying in more detail, using established and reliable research methods.

Soil microorganisms are considered key drivers for the maintenance of terrestrial ecosystems [34, 35]. Microbial biomass indices reflect the availability of C, N, S, and P in soil [36, 37]. Consequently, they are key components for assessing soil fertility and quality [38, 39] in different agricultural management systems [13, 28, 38, 40]. Soil quality is defined as the capacity of a soil to function within ecosystem to maintain key biological functions, environmental quality, and promote plant and animal health [38, 41]. This concept reflects the complexity and site-specificity of terrestrial ecosystems and the multiple linkages occurring between soil functions and soil-based ecosystem services [41]. Therefore, the evaluation of biological indicators of soil quality is necessary to link the microbial community composition and activity to the changes in the soil abiotic properties [39]. Multi-substrate induced respiration (multi-SIR) is a sensitive method used to determine the catabolic fingerprint of microbial communities [42]. Briefly, low molecular weight organic substances are added to the soil, and microbial utilization is measured by CO_2 release.

The long-term trial was established in 1993 near Bonn, Germany, to investigate the effects of biodynamic compost preparations after 8 years [33]. In this study, the application of FYM resulted in strong positive effects on SOC and microbial biomass C (MBC), whereas the biodynamic preparation had no significant additional effects with the only exception of decomposition activity. After 100 days, the decomposition of cotton strips buried in soil was significantly faster in plots that received FYM with all 6 recommended preparations than in plots that received pure FYM or FYM solely with the *Achillea* preparation.

As the field experiment is still ongoing, the opportunity was taken to resample all treatments in 2020, with the objective of analyzing MBC and microbial biomass nitrogen (MBN), fungal ergosterol, as well as functional microbial diversity, using the multi-SIR approach. The underlying hypothesis was that multi-SIR is able to differentiate between the three FYM treatments, pure FYM, FYM + *Achillea* and FYM + all 6 preparations.

Materials and methods

Site description

The experimental site is located on the certified organic research farm Wiesengut of the University of Bonn in Hennef (50°48′ N, 7°17′ E, 65 m asl) Germany. The experimental farm is situated in the Sieg river valley on a loamy Fluvisol [43]. It has been under organic management practices since 1986. The annual average temperature and precipitation are 10.3 °C and 840 mm, respectively [44]. The long-term field trial was established in 1993. Since then, cattle manure from Wiesengut livestock has been collected for a 90-day composting process carried out each autumn. The first FYM composting began in November 1992 and all matured FYM compost was applied each following year between February and March. All FYM treatments applied previously to soil sampling in July 2020 contained similar amounts of total C (338 ± 16 g kg^{-1}), total N (27 ± 3 g kg^{-1}), total K (20 ± 2 g kg^{-1}), total P (4 ± 0.5 g kg^{-1}), dry mass (25 ± 2%), and pH (8.43 ± 0.1).

Six main crops were arranged for a yearly rotation, including grass-clover, potatoes, winter wheat, field bean, spring wheat, and winter rye with an under-sown mixture of grass and red clover. The biodynamic preparations for the compost consisted of six herbal substances added to the compost piles at a rate of 9–10 g each to about 1.5 t dairy manure [33] to improve the final quality product [18, 19].

Experimental design and treatments

Four treatments with six replications were arranged in a randomized block design: (1) control without FYM application, (2) pure FYM application, (3) FYM with *Achillea*, and (4) FYM with all six preparations. Before the conversion of the experimental site Wiesengut to organic farming in the mid-1980s, soil potassium levels were very low. Since the *Achillea* preparation is linked to potassium processes by its developer Steiner [45], this treatment was chosen independent of increased soil nutrient levels in later farm management. Agricultural practices, such as weed management, sowing, and soil preparation, were identical among all experimental plots. Three FYM piles were prepared following recommendations by Koepf et al. [46]. For the five solid biodynamic applications 502 to 506, 50-cm-deep holes were bored around the FYM pile, and each was poured separately. Each hole was closed again with dairy manure. The liquid valerian preparation 507 was poured in 8-L tap water and sprayed on top of the pile. The same amount of water was sprayed on the treatments without the valerian preparation. Windrows were covered with straw and composted for approximately 90 days (average over the years) under similar conditions before application. In experimental plots of 60 m^2 (6 × 10 m), FYM was manually applied according to the treatments at the rate of 30 t ha^{-1} a^{-1} (≈ 25% dry mass).

Sampling

Six replicate soil samples per plot were collected from each useful area of the 24 plots (3 × 8 m) under spring wheat in July 2020. Samples were taken at 0–10 cm depth (higher microbial activity than deeper layers [47, 48]), using steel rings with a 9.7 cm inner diameter and 10 cm height. Prior to analysis, soil samples were sieved (< 2 mm) and stored in polyethylene bags at 4 °C.

General soil properties

Soil pH was measured with a glass electrode using a 1:2.5 soil-to-water (w/v) ratio. For soil texture, soil organic matter was first removed with 10% H_2O_2, followed by the addition of 0.4 M $Na_4P_2O_7$ to ensure effective microaggregates dispersion [49]. Sand determination was done by wet-sieving and gravitational separation for silt and clay contents [50, 51]. Carbonate content [52] was determined using a Scheibler apparatus (Calcimeter Bernard, Prolabo, Paris, France) [51]. Total carbon and nitrogen were obtained using a Vario MAX CN analyzer

(Elementar, Hanau, Germany). Soil organic carbon (SOC) was calculated as total C minus carbonate C [53, 54]. Water holding capacity (WHC) was determined using glass tubes with 30 mL capacity and a porous membrane at the bottom [55].

General microbial properties

Prior to analysis for microbial soil properties, soil samples were adjusted to 50% WHC and pre-incubated for seven days at 22 °C in the dark. Basal respiration was determined by incubating 50 g soil (oven-dry mass basis) in sealed and airtight jars [56]. CO_2 evolved from microbial activity was trapped in 5 mL 0.5 M NaOH. Solutions were titrated with 0.5 M HCl after adding saturated $BaCl_2$ solution [28]. The rest of the microbial properties were measured from incubated soil for basal respiration.

MBC and MBN were determined by chloroform-fumigation extraction (CFE) [57, 58]. Soil samples were split into two parts (10 g of moist soil each) for fumigated and non-fumigated treatments. Soil samples for fumigation were placed in a desiccator with chloroform for 24 h at room temperature. Samples from both treatments were extracted with 40 mL K_2SO_4 (0.5 M) by 30 min horizontal shaking at 200 rev min^{-1} and filtered (3-HW, Sartorius Stedim Biotech, Göttingen, Germany). The content of organic C and total N in the extracts was measured by using a Multi N/C 2100 S analyzer (Analytik Jena, Germany). MBC was calculated as E_C/k_{EC}, where E_C is the difference between organic C extracted from fumigated and non-fumigated soil, and k_{EC} is 0.45 [59]. MBN was calculated as E_N/k_{EN}, where E_N is the difference between total N extracted from fumigated and non-fumigated soil, and k_{EN} is 0.54 [60].

Ergosterol was extracted from 2 g moist soil with 100 mL ethanol by 30 min horizontal shaking at 250 rev min^{-1} [61] and quantified by reversed phase HPLC, using 100% methanol as mobile phase and a detection wavelength of 282 nm [62, 63].

Multi-substrate induced respiration (multi-SIR)

Multi-SIR was determined using the MicroResp™ method [64, 65]. Soil samples were adjusted to 35% WHC, soil with a higher WHC than required being air-dried in large trays at 15 °C until 35% WHC was reached. Into each 1.1 mL well of a deep-well microtiter plate (Nunc, Thermo Electron, Langenselbold, Germany), 300 mg were placed and incubated for 7 days in the dark at 25 °C prior to multi-SIR analysis. The physiological profiles were determined by adding aqueous solutions of 17 different C sources, including six amino acids (γ-aminobutyric acid (GABA), L-serine (Ser), L-alanine (Ala), L-cysteine (Cys), L-glutamine (GluN), and L-leucine (Leu)), three amino sugars (N-acetyl-glucosamine (NAG), D-glucosamine (GlcN), and D-galactosamine (GalN)), four sugars (L-arabinose (Ara), D-galactose (Gal), D-glucose (Glc), and D-fructose (Fru)), sugar alcohol sorbitol (Sor), phenolic organic acid protocatechuic acid (ProCa), two carboxylic acids (malic acid (MA) and citric acid (CA)), and distilled water (≈ basal respiration). Substrates were selected according to previous studies [28, 40, 47, 65] and considered as relevant as plant root exudates [66, 67] and metabolites [68, 69].

The MicroResp system consists of two deep-microplates (96 wells) placed face to face, one plate holding the soil samples with the carbon source and the other plate containing a gel with pH indicator dye [64]. A silicone rubber gasket with interconnecting holes between the corresponding wells allows the CO_2 evolved to be trapped in the detection gel. C source solutions were prepared by dissolving substrates at a concentration of 8 mg g^{-1} dry soil and considering 20 µl of solution in each well. Due to the lower solubility of Cys, GluN, Leu, and ProCa, solution concentrations were reduced to 4, 2, 1.3, and 0.8 mg g^{-1} soil, respectively. Gel plates were stored in dark plastic bags with wet paper towels and soda lime to avoid desiccation or atmospheric CO_2 reaction.

C sources were added to the soil (20 µl per well) and immediately sealed. A microtiter plate reader was used for colorimetric detection of the gel plates and measured before (T0) and after 4 h (T1) incubation at 25 °C and CO_2-trap absorbance of 572 nm (FLUOstar, BMG, Offenburg, Germany). Before measuring CO_2 evolution with the MicroResp™, the CO_2 trap is calibrated [40], and the calculation is performed as $\mu l\ CO_2 = 51 \times (0.2 + ABS)^3$, where ABS is the absorption difference between T1 and T0.

Statistical analysis

Data are presented as arithmetic mean on a dry weight basis. Data were tested for normal distribution using the Shapiro–Wilk's test and for homogeneity of variances via Levene's test. Transformation data were done if required, and non-transformed data were used to represent results in graphs. The significance of treatments effect was analyzed by a two-way ANOVA with block and treatment as factors, followed by Tukey´s Honestly Significant Difference (HSD) test to compare treatments. Differences between the mean of the three FYM treatments and the non-amended control were assessed using an independent sample t-test. Discriminant function analysis was conducted on the multi-SIR data for all samples, using SPSS 16.0 statistical software (SPSS 16.0).

Table 1 Soil texture and soil chemical parameters as affected by different farmyard cattle manure applications over 27 years. Long-term field experiment Wiesengut

Treatment	Sand (%)	Silt	Clay	Soil pH (H_2O)	Extractable P ($\mu g\ g^{-1}$ soil)	Extractable K
Control	43.9	45.6	10.4	6.63	34 b	104 b
FYM	45.6	44.3	10.1	6.70	45 a	171 ab
FYM + *Achillea*	47.1	42.9	10.0	6.60	38 ab	134 ab
FYM + all preparations	46.1	43.8	10.8	6.69	45 a	197 a
CV (±%)	7.3	7.8	13	2.1	16	34

CV = mean coefficient of variation between replicate plots ($n = 6$); different letters within a column represent a significant difference ($p < 0.05$; Tukey test)

Table 2 Soil organic carbon (SOC), microbial biomass carbon (MBC), ergosterol, basal respiration, ratio of MBC/SOC and ergosterol/MBC, and the metabolic quotient qCO_2, as affected by different farmyard cattle manure applications over 27 years. Long-term field experiment Wiesengut

Treatment	SOC ($mg\ g^{-1}$ soil)	MBC ($\mu g\ g^{-1}$ soil)	Ergosterol	CO_2 (μg C g^{-1} soil d^{-1})	MBC/SOC (%)	Ergosterol/MBC	qCO_2 (mg CO_2-C g^{-1} MBC d^{-1})
Control	9.1 b	210 b	0.29	6.65	2.30	0.15	30 a
FYM	10.1 a	265 a	0.26	5.97	2.63	0.10	22 ab
FYM + *Achillea*	9.9 a	270 a	0.32	5.53	2.73	0.12	18 b
FYM + all preparations	9.4 ab	229 ab	0.32	5.92	2.44	0.15	27 ab
CV (±%)	6.0	2.8	14	5.5	12	27	26

CV = mean coefficient of variation ($n = 6$); different letters within a column represent a significant difference ($p < 0.05$, Tukey test)

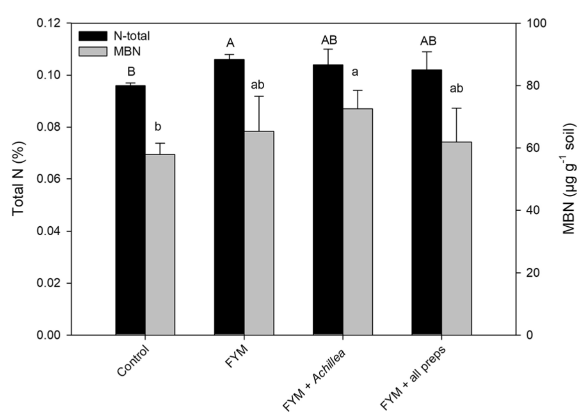


Fig. 1 Concentration of total N and microbial biomass nitrogen (MBN) in soil samples from experimental plots with four FYM treatments over 27 years. Long-term field experiment Wiesengut. Different letters on top of the bars represent significant differences from other treatments (Tukey's test)

Results

General soil and microbial properties

Soil texture and pH did not differ between the treatments (Table 1). The three FYM treatments, in comparison with the control treatment, significantly increased ($p < 0.05$, t-test) extractable P [+9 $\mu g\ g^{-1}$ soil], extractable K [+63 $\mu g\ g^{-1}$ soil], SOC [+0.7 $mg\ g^{-1}$ soil], MBC [+45 $\mu g\ g^{-1}$ soil] (Table 2), total N [+80 $\mu g\ g^{-1}$ soil], and MBN [+9 $\mu g\ g^{-1}$ soil], but decreased basal respiration [-0.84 $\mu g\ CO_2$-C g^{-1} soil d^{-1}], and metabolic quotient qCO_2 [-8 mg CO_2C g^{-1} MBC d^{-1}] (comparison of the single treatments with Tukey test in Table 2 and Fig. 1). In contrast, the ergosterol content remained generally unaffected by FYM incorporation. The mean SOC/total N ratio varied around 9.6 and the mean MB-C/N ratio around 4.1, without any FYM treatment effect. The three FYM treatments, in contrast to the control, increased but not significantly the contribution of MBC to SOC 2.3 to 2.6% and MBN to total N from 6.0 to 6.4%, whereas the contribution of ergosterol to MBC was decreased from 0.15 to 0.12%. Among FYM treatments, biodynamic preparations had no specific further significant effects on any general soil chemical and microbial properties.

Averaging all four treatments, the mean water-induced basal respiration of the multi-SIR-approach was 1.05 µl $CO_2\ g^{-1}\ h^{-1}$ (=13.5 $\mu g\ g^{-1}$ soil d^{-1}), approximately 125% higher than the mean basal respiration of the static microcosm approach (Fig. 2a; Table 2). The two activity indices were not significantly correlated ($r = -0.20$, $p = 0.4$). The mean MBC-SIR of all four treatments was

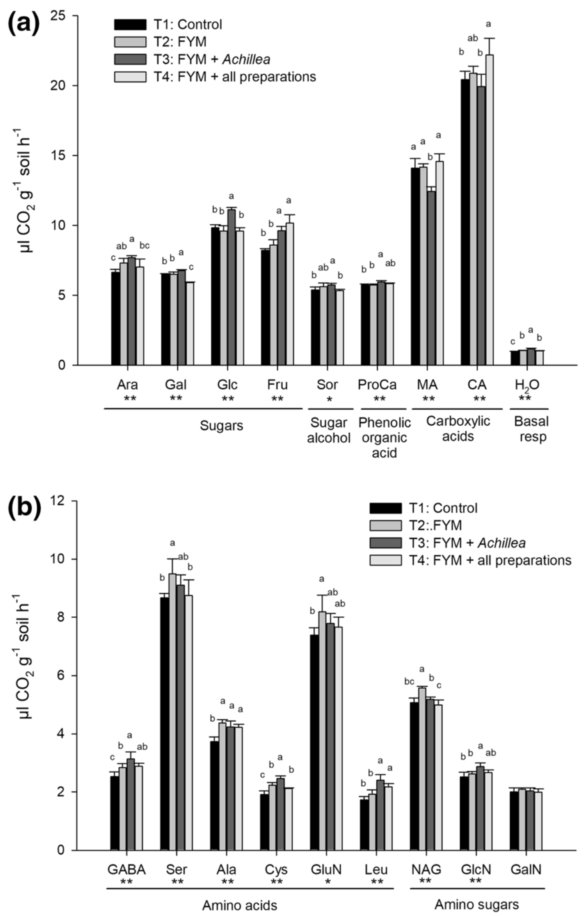


Fig. 2 **a** Mean catabolic response for sugars (L-arabinose (Ara), D-galactose (Gal), D-glucose (Glc), and D-fructose (Fru)), sugar alcohol sorbitol (Sor), phenolic organic acid protocatechuic acid (ProCa), carboxylic acids (malic acid (MA) and citric acid (CA)), and distilled water (≈ basal respiration), in experimental plots with four FYM treatments over 27 years. Long-term field experiment Wiesengut. Mean ± standard deviation. Different letters on top of the bars represent significant differences from other treatments (Tukey's test) **b** Catabolic response for amino acids (γ-aminobutyric acid (GABA), L-serine (Ser), L-alanine (Ala), L-cysteine (Cys), L-glutamine (GluN), and L-leucine (Leu)), and amino sugars (N-acetyl-glucosamine (NAG), D-glucosamine (GlcN), and D-galactosamine (GalN)) in experimental plots with four FYM treatments over 27 years. Long-term field experiment Wiesengut. Mean ± standard deviation, $t = 4$, $r = 6$, $n = 24$. Different letters on top of the bars represent significant differences from other treatments (Tukey's test)

301.09 µg g^{-1} soil, calculated according to Kaiser et al. [70] based on Glc-induced respiration (30 × 10.04 µl CO_2 g^{-1} h^{-1}), i.e., approximately 24% higher than the mean MBC concentration obtained by the CFE method (Fig. 2a; Table 2).

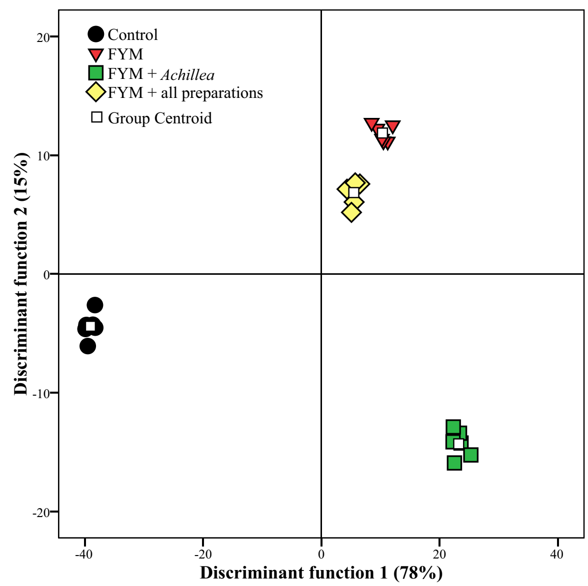


Fig. 3 Discriminant function analysis based on the catabolic response of 17 substrates and H_2O for the experimental plots with four FYM treatments applied over 27 years: no FYM (Control), pure FYM, FYM with *Achillea*, and FYM with all preparations. Long-term field experiment Wiesengut

Microbial functional diversity

The lowest respiratory responses were exhibited after the addition of the amino acids GABA, Cys, Leu, and the amino sugars GlcN and GalN, all ranging from 1.57 to 3.5 µl CO_2 g^{-1} soil h^{-1}, averaging all treatments (Fig. 2b). The highest mean respiration rates were measured after applying malic and citric acid (13.82 and 20.86 µl CO_2 g^{-1} soil h^{-1}, respectively, Fig. 2a). All substrates added, except GalN, were significantly affected by the three FYM treatments, in most cases increased.

Averaging all 17 substrates added, the FYM + *Achillea* treatment showed the highest mean respiratory response, decreasing in the order FYM + all preparations > FYM > control (6.97, 6.95, 6.93, and 6.61 µl CO_2 g^{-1} soil h^{-1}, respectively). In the FYM + *Achillea* treatment, the addition of GABA, Cys, Leu, GlcN, Ara, Gal, Glc, Sor, and ProCa exhibited the highest respiratory response, whereas MA and CA led to the lowest response. The addition of Ser, Ala, GluN, and NAG attained the highest respiratory response in the pure FYM treatment. The addition of MA and CA led to the highest respiratory response in the FYM + all preparations treatment (Fig. 2a, b).

Discriminant function 1 clearly separated the FYM treatments from the non-amended control (Fig. 3).

Discriminant function 2 separated all three FYM treatments from each other, with largest distance between the pure FYM treatment and the FYM + *Achillea* treatment.

Discussion

General soil and microbial properties

The 27-year field experiment at Wiesengut, Hennef, is especially suitable for investigating the effects of biodynamic preparations, as the soil of the treatment plots did not differ in texture or in soil pH. These two soil properties have dominating effects on soil microorganisms and often override subtle treatments effects, even in the long term. This is a serious problem in the DOK experiment [71] and in the Darmstadt long-term fertilization experiment [62]. The mean pH has increased by 1.41 units in the topsoil since 2001 [33] by liming. This has general positive effects on soil microorganisms [72, 73] and SOC sequestration by supporting the formation of Ca–humus bridges [74].

Increases of SOC, MBC, total N, and MBN in the three FYM treatments, in contrast to the control, are in line with the positive effects of FYM incorporation usually observed in arable soils [20, 75–77]. The same is true for increased MBC/SOC and MBN/total N ratios, indicating an increased SOM availability to soil microorganisms in FYM amended soils [33, 78, 79]. The low MB-C/N ratios observed in all treatments indicate sufficient N and P availability for a generally C-limited soil microbial community [28]. The MBC/SOC ratios and qCO_2 were negatively correlated ($r = -0.43$, $p < 0.05$), as repeatedly observed [80–82], demonstrating the inverse relationship between anabolism and catabolism, caused by management-induced shifts in maintenance energy requirements of soil microbial communities [83]. The current qCO_2 values were in the range obtained by Mäder et al. [13], whereas those obtained by Zaller and Köpke [33] were considerably higher, due to a 4-times higher mean basal respiration.

In contrast, the mean MBC contents, obtained by the SIR method, varied around 301 µg g^{-1} soil, i.e., between the current CFE (Table 2) and SIR data. However, the similarity between these MBC estimates is remarkable, considering the difference in methodological approaches. This gives confidence in the general reliability of the multi-SIR approach. In contrast, basal respiration exhibits considerably more variation, as this important index is more strongly affected by measurement details, i.e., pretreatment, length of incubation period, and CO_2 measurement system [40, 84].

The absence of positive effects of the three FYM treatments on the fungal cell-membrane ergosterol and on the ergosterol/MBC ratio was previously observed in the Darmstadt long-term fertilization experiment. FYM incorporation increased bacterial biomass in particular, due to the heat period during composting of the feces/straw mixture [20]. In arable and grassland soils, ergosterol is a highly specific indicator for the contribution of saprotrophic fungi to the soil microbial biomass [85, 86]. The relatively low ergosterol/MBC ratio may suggest a strong presence of arbuscular mycorrhizal fungi, which do not contain ergosterol [85]. The absence of a correlation between the ergosterol/MBC and MB-C/N ratios ($r = -0.35$, $p = 0.1$) support the view that the contribution of saprotrophic fungi to the microbial community is not reflected by the MB-C/N ratio [28].

Multi-SIR approach and nutrient cycling

In most studies, usually no effects of biodynamic preparations were reported, including plant physiological performance [87], soil fertility [21], or microbial biomass and activity indicators [20]. This is in line with the current result that biodynamic preparations had no discernible effects on general soil and microbial properties. In contrast, the current multi-SIR data not only revealed significant general effects of biodynamic preparations, but were also able to differentiate between the application of FYM + *Achillea* and the standard application of FYM + all preparations. The application of FYM + *Achillea* showed a stronger ability to improve the N status of the microbial community in comparison with FYM + all preparations. This is indicated by the higher catabolic use of glucosamine as well as the amino acids γ-aminobutyric acid, cysteine, and leucine (Fig. 2b). In contrast, N depletion is indicated by a lower respiratory response to N-containing substrates, as they are used for anabolic growth processes by soil microorganisms [47].

The highest respiratory responses were obtained in carboxylic acids and the lowest in amino acids (except Ser and GluN) and amino sugars, which is in line with results from Sradnick et al. [40], with the highest and lowest values in the same group of substrates. Nevertheless, they reported roughly 70% lower respiratory responses to the respective substrates, due to the markedly lower MBC contents in the Darmstadt long-term fertilization trial. Fritz et al. [28] investigated alkaline vineyard soils and used similar substrates to those in the current study. They observed no biodynamic preparation effects on Ala, GluN, and GlcN, and the highest respiratory response to Ser, Fru, and Glc addition. The respiratory responses were on average roughly 50% lower due to the alkaline pH, trapping partly the CO_2 evolved during the short incubation period [40, 88]. The higher the respiration rate, the lower the anabolic demand for a particular substrate, indicating the C abundance of the soil microorganisms [28].

The patterns of discrimination between treatments were found to be generally repeatable observed by the close proximity of variate scores from each treatment (Fig. 3). The reasons for the effect of biodynamic preparation are still a matter of considerable debate [15, 20, 24]. Although these preparations contain bio-stimulating compounds [31, 32], they could hardly explain their effects, as they are sprayed and added in highly diluted concentrations on compost, plants, and soil [33]. In the laboratory, application of low concentrated soluble substances to soil caused a strong respiratory response, considerably exceeding the amount of substrate added [89]. However, this effect has not been observed under field conditions until now. Another explanation for biodynamic preparation effects might be that their application under practical farming conditions is changing attitude and awareness of farmers to their management practices [20, 28, 90]. However, such indirect effects seem to be unlikely in replicated field experiments with small plots, such as the current study.

Conclusions

The three composted FYM treatments, contrasted with the control without FYM application, revealed generally positive effects on all general soil chemical (SOC, total N) and biological properties (basal respiration, MBC, MBN) measured in the current study, except fungal ergosterol. After 27 experimental years, discriminant function analysis of multi-SIR data not only revealed significant general effects of FYM incorporation, but was also able to differentiate between the sole application of the FYM + *Achillea* and the application of FYM + all preparations. The FYM + *Achillea* treatment was specifically able to improve the N status of the microbial community, as indicated by the higher catabolic use of glucosamine as well as the amino acids γ-aminobutyric acid, cysteine, and leucine. The reason for different effects of the sole *Achillea* preparation in comparison with all 6 preparations cannot be explained by the current study.

Abbreviations

FYM: Composted farmyard manure; MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen; multi-SIR: Multi-substrate-induced respiration; SOC: Soil organic carbon; SOM: Soil organic matter; WHC: Water holding capacity. Substrates: GABA: γ-aminobutyric acid; Ser: L-Serine; Ala: L-Alanine; Cys: L-Cysteine; GluN: L-Glutamine; Leu: L-Leucine; NAG: N-Acetyl-glucosamine; GlcN: D-Glucosamine; GalN: D-Galactosamine; Ara: L-Arabinose; Gal: D-Galactose; Glc: D-Glucose; Fru: D-Fructose; Sor: Sorbitol; ProCa: Protocatechuic acid; MA: Malic acid; CA: Citric acid.

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Author contributions

Field experiment was designed and established by UK and over the years operated by CD. HR carried out the laboratory analyses, interpreted the results, and wrote the first draft. JF and RJ interpreted the results, finalized and reviewed the manuscript with UK. All authors read and approved the final manuscript.

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Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Menšík L, Hlisnikovský L, Pospíšilová L, Kunzová E. The effect of application of organic manures and mineral fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. J Soil Sediment. 2018;18:2813–22. https://doi.org/10.1007/s11368-018-1933-3.
2. Hati KM, Mandal KG, Misra AK, Ghosh PK, Bandyopadhyay KK. Effect of inorganic fertilizer and farmyard manure on soil physical properties, root distribution, and water-use efficiency of soybean in Vertisols of central India. Bioresource Technol. 2006;97:2182–8. https://doi.org/10.1016/j.biortech.2005.09.033.
3. Gross A, Glaser B. Meta-analysis on how manure application changes soil organic carbon storage. Sci Rep. 2021;11:5516. https://doi.org/10.1038/s41598-021-82739-7.
4. Amelung W, Bossio D, de Vries W, Kögel-Knabner I, Lehmann J, Amundson R, et al. Towards a global-scale soil climate mitigation strategy. Nat Commun. 2020;11:5427. https://doi.org/10.1038/s41467-020-18887-7.
5. Bhardwaj AK, Rajwar D, Mandal UK, Ahamad S, Kaphaliya B, Minhas PS, et al. Impact of carbon inputs on soil carbon fractionation, sequestration and biological responses under major nutrient management practices for rice-wheat cropping systems. Sci Rep. 2019;9:9114. https://doi.org/10.1038/s41598-019-45534-z.

6. Breyer C, Fasihi M, Bajamundi C, Creutzig F. Direct air capture of CO_2: a key technology for ambitious climate change mitigation. Joule. 2019;3:2053–7. https://doi.org/10.1016/j.joule.2019.08.010.
7. Stockmann U, Adams MA, Crawford JW, Field DJ, Henakaarchchi N, Jenkins M, et al. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agr Ecosyst Environ. 2013;164:80–99. https://doi.org/10.1016/j.agee.2012.10.001.
8. Di Sacco A, Hardwick KA, Blakesley D, Brancalion PHS, Breman E, Cecilio Rebola L, et al. Ten golden rules for reforestation to optimize carbon sequestration, biodiversity recovery and livelihood benefits. Global Change Biol. 2021;27:1328–48. https://doi.org/10.1111/gcb.15498.
9. Wang J, Wang K, Wang X, Ai Y, Zhang Y, Yu J, et al. Carbon sequestration and yields with long-term use of inorganic fertilizers and organic manure in a six-crop rotation system. Nutr Cycl Agroecosys. 2018;111:87–98. https://doi.org/10.1007/s10705-018-9920-z.
10. Sriprapakhan P, Artkla R, Nuanual S, Maneechot P. Economic and ecological assessment of integrated agricultural bio-energy and conventional agricultural energy frameworks for agriculture sustainability. J Saudi Soc Agr Sci. 2021;20:227–34. https://doi.org/10.1016/j.jssas.2021.02.001.
11. Canwat V, Onakuse S. Organic agriculture: A fountain of alternative innovations for social, economic, and environmental challenges of conventional agriculture in a developing country context. Clean Circ Bioecon. 2022;3: 100025. https://doi.org/10.1016/j.clcb.2022.100025.
12. Knapp S, van der Heijden MGA. A global meta-analysis of yield stability in organic and conservation agriculture. Nat Commun. 2018;9:3632. https://doi.org/10.1038/s41467-018-05956-1.
13. Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U, et al. Soil fertility and biodiversity in organic farming. Science. 2002;296:1694–7. https://doi.org/10.1126/science.1071148.
14. Köninger J, Lugato E, Panagos P, Kochupillai M, Orgiazzi A, Briones MJ, et al. Manure management and soil biodiversity: Towards more sustainable food systems in the EU. Agr Syst. 2021;194: 103251. https://doi.org/10.1016/j.agsy.2021.103251.
15. Chalker-Scott L. The science behind biodynamic preparations: a literature review. Hortic Technol. 2013;23:814–9. https://doi.org/10.21273/horttech.23.6.814.
16. Turinek M, Grobelnik-Mlakar S, Bavec M, Bavec F. Biodynamic agriculture research progress and priorities. Renew Agr Food Syst. 2009;24:146–54. https://doi.org/10.1017/S174217050900252X.
17. Paull J, Hennig B. A world map of biodynamic agriculture. Agr Biol Sci J. 2020;6:114–9.
18. Reeve JR, Carpenter-Boggs L, Reganold JP, York AL, Brinton WF. Influence of biodynamic preparations on compost development and resultant compost extracts on wheat seedling growth. Bioresource Technol. 2010;101:5658–66. https://doi.org/10.1016/j.biortech.2010.01.144.
19. Steiner R. Agriculture: spiritual foundations for the renewal of agriculture. 1st ed. Hudson, New York: Anthroposophic Press; 1993.
20. Faust S, Heinze S, Ngosong C, Sradnick A, Oltmanns M, Raupp J, et al. Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization. Appl Soil Ecol. 2017;114:82–9. https://doi.org/10.1016/j.apsoil.2017.03.006.
21. Carpenter-Boggs L, Reganold JP, Kennedy AC. Biodynamic preparations: short-term effects on crops, soils, and weed populations. Am J Altern Agr. 2000;15:110–8. https://doi.org/10.1017/S0889189300008614.
22. Tassoni A, Tango N, Ferri M. Comparison of biogenic amine and polyphenol profiles of grape berries and wines obtained following conventional, organic and biodynamic agricultural and oenological practices. Food Chem. 2013;139:405–13. https://doi.org/10.1016/j.foodchem.2013.01.041.
23. Morrison-Whittle P, Lee SA, Goddard MR. Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems. Agr Ecosys Environ. 2017;246:306–13. https://doi.org/10.1016/j.agee.2017.05.022.
24. Soustre-Gacougnolle I, Lollier M, Schmitt C, Perrin M, Buvens E, Lallemand J-F, et al. Responses to climatic and pathogen threats differ in biodynamic and conventional vines. Sci Rep. 2018;8:16857. https://doi.org/10.1038/s41598-018-35305-7.
25. Villanueva-Rey P, Vázquez-Rowe I, Moreira MT, Feijoo G. Comparative life cycle assessment in the wine sector: biodynamic vs. conventional viticulture activities in NW Spain. J Clean Prod. 2014;65:330–41. https://doi.org/10.1016/j.jclepro.2013.08.026.
26. Juknevičienė E, Danilčenko H, Jarienė E, Živatkauskienė V, Zeise J, Fritz J, et al. The effect of biodynamic preparations on growth and fruit quality of giant pumpkin (*Cucurbita maxima* D.). Chem Biol Technol Agr. 2021. https://doi.org/10.1186/s40538-021-00258-z.
27. Fritz J, Döring J, Athmann M, Meissner G, Kauer R, Schultz HR, et al. Wine quality under integrated, organic and biodynamic management using image-forming methods and sensory analysis. Chem Biol Technol Agr. 2021. https://doi.org/10.1186/s40538-021-00261-4.
28. Fritz J, Jannoura R, Lauer F, Schenk J, Masson P, Joergensen RG, et al. Functional microbial diversity responses to biodynamic management in Burgundian vineyard soils. Biol Agric Hortic. 2020;36:172–86. https://doi.org/10.1080/01448765.2020.1762739.
29. Rodas-Gaitán HA, Palma-García JM, Olivares-Sáenz E, Gutiérrez-Castorena EV, Vázquez-Alvarado R. Biodynamic preparations on static pile composting from prickly pear cactus and moringa crop wastes. Open Agr. 2019;4:247–57. https://doi.org/10.1515/opag-2019-0023.
30. Rodas-Gaitán HA, Vázquez Alvarado RE, Olivares Sáenz E, Aranda Ruiz J, Palma García JM. Estabilidad de compostas estáticas biodinámicas a partir de restos de cultivos Regionales. Rev Mex Cienc Agr. 2019;10:187–95. https://doi.org/10.29312/remexca.v10i1.1337.
31. Giannattasio M, Vendramin E, Fornasier F, Alberghini S, Zanardo M, Stellin F, et al. Microbiological features and bioactivity of a fermented manure product (preparation 500) used in biodynamic agriculture. J Microbiol Biotechn. 2013;23:644–51. https://doi.org/10.4014/jmb.1212.12004.
32. Spaccini R, Mazzei P, Squartini A, Giannattasio M, Piccolo A. Molecular properties of a fermented manure preparation used as field spray in biodynamic agriculture. Environ Sci Pollut R. 2012;19:4214–25. https://doi.org/10.1007/s11356-012-1022-x.
33. Zaller J, Köpke U. Effects of traditional and biodynamic farmyard manure amendment on yields, soil chemical, biochemical and biological properties in a long-term field experiment. Biol Fert Soils. 2004;40:222–9. https://doi.org/10.1007/s00374-004-0772-0.
34. Singh JS, Gupta VK. Soil microbial biomass: a key soil driver in management of ecosystem functioning. Sci Total Environ. 2018;634:497–500. https://doi.org/10.1016/j.scitotenv.2018.03.373.
35. Li Y, Chang SX, Tian L, Zhang Q. Conservation agriculture practices increase soil microbial biomass carbon and nitrogen in agricultural soils: a global meta-analysis. Soil Biol Biochem. 2018;121:50–8. https://doi.org/10.1016/j.soilbio.2018.02.024.
36. Cleveland CC, Liptzin D. C:N: P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry. 2007;85:235–52. https://doi.org/10.1007/s10533-007-9132-0.
37. Heuck C, Weig A, Spohn M. Soil microbial biomass C:N: P stoichiometry and microbial use of organic phosphorus. Soil Biol Biochem. 2015;85:119–29. https://doi.org/10.1016/j.soilbio.2015.02.029.
38. Joergensen RG. Organic matter and micro-organisms in tropical soils. In: Dion P, editor. Soil biology and agriculture in the tropics. Berlin: Springer Berlin Heidelberg; 2010. p. 17–44.
39. Thioye B, Legras M, Castel L, Hirissou F, Chaftar N, Trinsoutrot-Gattin I, et al. Understanding arbuscular mycorrhizal colonization in walnut plantations: the contribution of cover crops and soil microbial communities. Agriculture. 2022;12:1. https://doi.org/10.3390/agriculture12010001.
40. Sradnick A, Murugan R, Oltmanns M, Raupp J, Joergensen RG. Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer. Appl Soil Ecol. 2013;63:23–8. https://doi.org/10.1016/j.apsoil.2012.09.011.
41. Bünemann EK, Bongiorno G, Bai Z, Creamer RE, de Deyn G, de Goede R, et al. Soil quality—a critical review. Soil Biol Biochem. 2018;120:105–25. https://doi.org/10.1016/j.soilbio.2018.01.030.
42. Tlili A, Marechal M, Montuelle B, Volat B, Dorigo U, Bérard A, et al. Use of the MicroResp™ method to assess pollution-induced community tolerance to metals for lotic biofilms. Environ Pollut. 2011;159:18–24. https://doi.org/10.1016/j.envpol.2010.09.033.
43. Sufyan M, Abbasi A, Dildar Gogi M, Arshad M, Nawaz A, Neuhoff D, et al. Efficacy of *Beauveria Bassiana* for the management of economically important wireworm species (Coleoptera: Elateridae) in organic farming. Gesunde Pflanz. 2017;69:197–202. https://doi.org/10.1007/s10343-017-0406-8.
44. Kemper R, Bublitz TA, Müller P, Kautz T, Döring TF, Athmann M, et al. Vertical root distribution of different cover crops determined with the profile

wall method. Agriculture. 2020;10:503. https://doi.org/10.3390/agriculture10110503.
45. Steiner R. Geisteswissenschaftliche Grundlagen zum Gedeihen der Landwirtschaft. 5th ed. Dornach: Rudolf-Steiner-Verlag; 1975.
46. Koepf HH, Pettersson BD, Schaumann W. Biologisch-dynamische Landwirtschaft. Eine Einführung. 4th ed. Stuttgart: Eugen Ulmet; 1980.
47. Struecker J, Joergensen RG. Microorganisms and their substrate utilization patterns in topsoil and subsoil layers of two silt loams, differing in soil organic C accumulation due to colluvial processes. Soil Biol Biochem. 2015;91:310–7. https://doi.org/10.1016/j.soilbio.2015.09.011.
48. Loeppmann S, Blagodatskaya E, Pausch J, Kuzyakov Y. Enzyme properties down the soil profile—a matter of substrate quality in rhizosphere and detritusphere. Soil Biol Biochem. 2016;103:274–83. https://doi.org/10.1016/j.soilbio.2016.08.023.
49. Jensen JL, Schjønning P, Watts CW, Christensen BT, Munkholm LJ. Soil texture analysis revisited: removal of organic matter matters more than ever. PLoS ONE. 2017;12: e0178039. https://doi.org/10.1371/journal.pone.0178039.
50. Hobley EU, Prater I. Estimating soil texture from vis-NIR spectra. Eur J Soil Sci. 2019;70:83–95. https://doi.org/10.1111/ejss.12733.
51. Blume H-P, Stahr K, Leinweber P. Bodenkundliches Praktikum. 3rd ed. Heidelberg: Spektrum Akademischer Verlag; 2011.
52. Asabere SB, Zeppenfeld T, Nketia KA, Sauer D. Urbanization leads to increases in pH, carbonate, and soil organic matter stocks of arable soils of Kumasi, Ghana (West Africa). Front Environ Sci. 2018. https://doi.org/10.3389/fenvs.2018.00119.
53. Mirzaeitalarposhti R, Demyan MS, Rasche F, Cadisch G, Müller T. Overcoming carbonate interference on labile soil organic matter peaks for midDRIFTS analysis. Soil Biol Biochem. 2016;99:150–7. https://doi.org/10.1016/j.soilbio.2016.05.010.
54. Wang X, Wang J, Xu M, Zhang W, Fan T, Zhang J, et al. Carbon accumulation in arid croplands of northwest China: pedogenic carbonate exceeding organic carbon. Sci Rep. 2015;5:11439. https://doi.org/10.1038/srep11439.
55. Hallam J, Hodson ME. Impact of different earthworm ecotypes on water stable aggregates and soil water holding capacity. Biol Fert Soils. 2020;56:607–17. https://doi.org/10.1007/s00374-020-01432-5.
56. Bolat İ. Microbial biomass, basal respiration, and microbial indices of soil in diverse croplands in a region of northwestern Turkey (Bartın). Environ Monit Assess. 2019;191:695. https://doi.org/10.1007/s10661-019-7817-1.
57. Brookes PC, Powlson DS, Jenkinson DS. Measurement of microbial biomass phosphorus in soil. Soil Biol Biochem. 1982;14:319–29. https://doi.org/10.1016/0038-0717(82)90001-3.
58. Joergensen RG, Anderson T-H, Wolters V. Carbon and nitrogen relationships in the microbial biomass of soils in beech (*Fagus sylvatica* L.) forests. Biol Fert Soils. 1995;19:141–7. https://doi.org/10.1007/BF00336150.
59. Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biol Biochem. 1990;22:1167–9. https://doi.org/10.1016/0038-0717(90)90046-3.
60. Brookes PC, Landman A, Pruden G, Jenkinson DS. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem. 1985;17:837–42. https://doi.org/10.1016/0038-0717(85)90144-0.
61. Djajakirana G, Joergensen RG, Meyer B. Ergosterol and microbial biomass relationship in soil. Biol Fert Soils. 1996;22:299–304. https://doi.org/10.1007/BF00334573.
62. Heinze S, Raupp J, Joergensen RG. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil. 2010;328:203–15. https://doi.org/10.1007/s11104-009-0102-2.
63. Linsler D, Taube F, Geisseler D, Joergensen RG, Ludwig B. Temporal variations of the distribution of water-stable aggregates, microbial biomass and ergosterol in temperate grassland soils with different cultivation histories. Geoderma. 2015;241–242:221–9. https://doi.org/10.1016/j.geoderma.2014.11.013.
64. Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl Environ Microb. 2003;69:3593–9. https://doi.org/10.1128/AEM.69.6.3593-3599.2003.
65. Creamer RE, Stone D, Berry P, Kuiper I. Measuring respiration profiles of soil microbial communities across Europe using MicroResp™ method. Appl Soil Ecol. 2016;97:36–43. https://doi.org/10.1016/j.apsoil.2015.08.004.
66. Meyer A, Fischer H, Kuzyakov Y, Fischer K. Improved RP-HPLC and anion-exchange chromatography methods for the determination of amino acids and carbohydrates in soil solutions. J Plant Nutr Soil Sc. 2008;171:917–26. https://doi.org/10.1002/jpln.200700235.
67. Kierul K, Voigt B, Albrecht D, Chen X-H, Carvalhais LC, Borriss R, et al. Influence of root exudates on the extracellular proteome of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Microbiology. 2015;161:131–47. https://doi.org/10.1099/mic.0.083576-0.
68. Singh R, Kumar M, Mittal A, Mehta PK. Microbial metabolites in nutrition, healthcare and agriculture. 3 Biotech. 2017;7:15. https://doi.org/10.1007/s13205-016-0586-4.
69. Tyc O, Song C, Dickschat JS, Vos M, Garbeva P. The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trends Microbiol. 2017;25:280–92. https://doi.org/10.1016/j.tim.2016.12.002.
70. Kaiser EA, Mueller T, Joergensen RG, Insam H, Heinemeyer O. Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. Soil Biol Biochem. 1992;24:675–83. https://doi.org/10.1016/0038-0717(92)90046-Z.
71. Fließbach A, Oberholzer H-R, Gunst L, Mäder P. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agr Ecosyst Environ. 2007;118:273–84. https://doi.org/10.1016/j.agee.2006.05.022.
72. Das S, Jeong ST, Das S, Kim PJ. Composted cattle manure increases microbial activity and soil fertility more than composted swine manure in a submerged rice paddy. Front Microbiol. 2017;8:1702. https://doi.org/10.3389/fmicb.2017.01702.
73. Fuentes JP, Bezdicek DF, Flury M, Albrecht S, Smith JL. Microbial activity affected by lime in a long-term no-till soil. Soil Till Res. 2006;88:123–31. https://doi.org/10.1016/j.still.2005.05.001.
74. Vogel S, Bönecke E, Kling C, Kramer E, Lück K, Nagel A, et al. Base neutralizing capacity of agricultural soils in a quaternary landscape of north-east Germany and its relationship to best management practices in lime requirement determination. Agronomy. 2020;10:877. https://doi.org/10.3390/agronomy10060877.
75. Ren F, Sun N, Xu M, Zhang X, Wu L, Xu M, et al. Changes in soil microbial biomass with manure application in cropping systems: a meta-analysis. Soil Till Res. 2019;194: 104291. https://doi.org/10.1016/j.still.2019.06.008.
76. Böhme L, Langer U, Böhme F. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agr Ecosyst Environ. 2005;109:141–52. https://doi.org/10.1016/j.agee.2005.01.017.
77. Maharjan M, Sanaullah M, Razavi BS, Kuzyakov Y. Effect of land use and management practices on microbial biomass and enzyme activities in subtropical top-and sub-soils. Appl Soil Ecol. 2017;113:22–8. https://doi.org/10.1016/j.apsoil.2017.01.008.
78. Liu E, Yan C, Mei X, He W, Bing SH, Ding L, et al. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. Geoderma. 2010;158:173–80. https://doi.org/10.1016/j.geoderma.2010.04.029.
79. Kumar U, Shahid M, Tripathi R, Mohanty S, Kumar A, Bhattacharyya P, et al. Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. Ecol Indic. 2017;73:536–43. https://doi.org/10.1016/j.ecolind.2016.10.014.
80. Martín-Lammerding D, Navas M, Del Albarrán MM, Tenorio JL, Walter I. LONG term management systems under semiarid conditions: Influence on labile organic matter, β-glucosidase activity and microbial efficiency. Appl Soil Ecol. 2015;96:296–305. https://doi.org/10.1016/j.apsoil.2015.08.021.
81. Frimpong KA, Abban-Baidoo E, Marschner B. Can combined compost and biochar application improve the quality of a highly weathered coastal savanna soil? Heliyon. 2021;7(5):E07089. https://doi.org/10.1016/j.heliyon.2021.e07089.
82. Toh FA, Ndam LM, Angwafo TE, Christopher N. Effect of land use management patterns on mineralization kinetics of soil organic carbon in Mount Bambouto Caldera Area of Cameroon. Open J Soil Sci. 2020;10:391–409. https://doi.org/10.4236/ojss.2020.109021.
83. Anderson T-H, Domsch KH. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping

histories. Soil Biol Biochem. 1990;22:251–5. https://doi.org/10.1016/0038-0717(90)90094-G.
84. Sradnick A, Oltmanns M, Raupp J, Joergensen RG. Microbial biomass and activity down the soil profile after long-term addition of farmyard manure to a sandy soil. Org Agr. 2018;8:29–38. https://doi.org/10.1007/s13165-016-0170-6.
85. Joergensen R, Wichern F. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol Biochem. 2008;40:2977–91. https://doi.org/10.1016/j.soilbio.2008.08.017.
86. Bencherif K, Boutekrabt A, Fontaine J, Laruelle F, Dalpè Y, Sahraoui AL-H, et al. Impact of soil salinity on arbuscular mycorrhizal fungi biodiversity and microflora biomass associated with *Tamarix articulata* Vahll rhizosphere in arid and semi-arid Algerian areas. Sci Total Environ. 2015;533:488–94. https://doi.org/10.1016/j.scitotenv.2015.07.007.
87. Döring J, Frisch M, Tittmann S, Stoll M, Kauer R. Growth, yield and fruit quality of grapevines under organic and biodynamic management. PLoS ONE. 2015;10: e0138445. https://doi.org/10.1371/journal.pone.0138445.
88. Oren A, Steinberger Y. Coping with artifacts induced by $CaCO_3$–CO_2–H_2O equilibria in substrate utilization profiling of calcareous soils. Soil Biol Biochem. 2008;40:2569–77. https://doi.org/10.1016/j.soilbio.2008.06.020.
89. de Nobili M, Contin M, Mondini C, Brookes P. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol Biochem. 2001;33:1163–70. https://doi.org/10.1016/S0038-0717(01)00020-7.
90. Patzel N, Sticher H, Karlen DL. Soil fertility—phenomenon and concept. J Plant Nutr Soil Sc. 2000;163:129–42. https://doi.org/10.1002/(SICI)1522-2624(200004)163:2%3c129::AID-JPLN129%3e3.0.CO;2-D.

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