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Enhancing Greenhouse Soil Quality Through Ecological Intensification (EI). A Case Study

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Intensive greenhouse cultivation, characterized by high agrochemical inputs and minimal organic amendments, maximizes crop productivity but often leads to soil degradation and environmental harm, notably through nitrate leaching and increased nitrous oxide (N_2O) emissions. To reduce agricultural inputs that may lead to soil degradation, this study evaluates an alternative fertilization strategy based in ecological intensification (EI). Specifically, a management system incorporating horticultural crop residues and organic amendments—with limited use of inorganic fertilizers—was compared to a conventional fertilization system (C) over a six-year period. Soil quality was assessed using physical and chemical indicators alongside microbial gene abundance (16s, ITS) and genes related to denitrification processes (*nirK*, *nirS*, *nosZ*1, and *nosZ*2) measured by Real-Time PCR. The EI system enhanced soil organic matter and soil structure by enhancing macroporosity and aggregate stability. However, it also increased the risk of salinization. Fungal abundance and the key denitrification genes (*nosZ1* and *nosZ2*) were significantly higher under EI management. The fungal-to-bacterial ratio approached, but did not reach, statistical significance, and the *nos/nir* gene ratio—an indirect indicator of N_2O emission potential—remained similar between treatments. These findings suggest a complex interaction between soil quality and denitrifier community dynamics that warrants further investigation, particularly to assess potential N_2O emissions.

Keywords: denitrification, ecological management, *nirK*, *nirS*, *nosZ*

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INTRODUCTION

Sustainable soil management remains a critical global challenge (Obade, 2019; FAO and ITPS, 2015). Considerable research has focused on cropping practices that enhance soil quality (Cuevas et al., 2019; Urra et al., 2019; Bai et al., 2018; Oenema et al., 2017). However, in intensive vegetable production systems situated in warm climates—such as those along the Mediterranean coast—soil management practices are often suboptimal. These systems are typically characterized by limited crop rotation, heavy irrigation, and intensive application of mineral fertilizers, particularly nitrogen (N), while receiving little to no organic matter inputs (Granados et al., 2013; Gallardo et al., 2011).

The province of Almería in southeastern Spain exemplifies this model. Covering approximately 32.368 ha, it hosts the highest concentration of greenhouses in the country. Most of these greenhouses are low- to medium-cost structures with plastic coverings, used to cultivate horticultural crops under semi-arid conditions. This intensive production system has long been associated with soil degradation (in the sense described by Vereecken et al., 2016) and a decline in soil quality (Norris and Congreves, 2018; Yazdanpanah et al., 2016). Furthermore, degradation of soil health often accelerates the deterioration of related environmental systems, such as groundwater resources (Bonachela et al., 2018; Cabrera et al., 2016). Inefficient fertilization practices in the region also contribute to nitrogen losses via leaching and denitrification, leading to elevated emissions of nitrous oxide (Seitzinger and Phillips, 2017).

To counter these adverse effects, a range of restorative strategies based on ecological intensification have been proposed (Bender et al., 2016). These include leveraging endogenous biological resources, reducing agrochemical inputs—aligned with the European Union's "Farm to Fork" strategy—and transitioning toward organic management approaches. One such practice is the integration of cover crops, which have demonstrated positive effects on several dimensions of greenhouse soil quality (Del Moral et al., 2012).

Numerous studies have proposed physical, chemical, biological indicators, or composite indices to evaluate soil quality (Juhos et al., 2019; Li et al., 2019; Obade and Lal, 2016). Nonetheless, the selection of appropriate indicators and interpretation of measurements are complicated by the inherent complexity and site-specific nature of soils (Ghiglieno et al., 2019; Bünemann et al., 2018). Therefore, careful selection of soil quality indicators must consider: (i) the temporal responsiveness of indicators to management changes, and (ii) the specific soil functions or threats they are meant to assess.

In the context of greenhouse horticulture in Almería, the main soil threats include salinization, compaction, loss of organic matter, and biodiversity decline (Salinas et al., 2020). Soil electrical conductivity (EC), determined using various soil-to-water ratios, is widely recognized as a reliable indicator of salinization (Lin and Bañuelos, 2015). For soil compaction, the percentage of water-stable aggregates following dispersant treatment serves as a robust metric. To assess organic matter depletion, soil organic carbon (SOC) is the most frequently employed indicator. However, total SOC changes are often subtle and slow to manifest; thus, evaluating rapidly cycling carbon pools, such as water-soluble organic carbon (WSOC), can provide a more sensitive measure of short-term changes in soil carbon dynamics (Bongiorno et al., 2019; Wander and Bidart, 2000).

Biological indicators also play a central role in soil quality assessment. The fungal-to-bacterial rate is a widely accepted microbial metric and has been linked to shifts in carbon cycling dynamics (Malik et al., 2016). The abundance of microbial groups is commonly quantified through real-time PCR, targeting 16S rRNA genes in prokaryotes (Hill et al., 2000) and internal transcribed spacer (ITS1 and ITS2) regions in fungi (Lauber et al., 2008).

Denitrification, a key microbial process contributing to nitrogen losses and greenhouse gas emissions, is typically evaluated by quantifying the abundance of functional genes. The *nirK* and *nirS* genes encode distinct nitrite reductase enzymes, while the *nosZ* gene encodes nitrous oxide reductase, the only known enzyme that converts N_2O to inert N_2 gas (Kuypers et al., 2018). Notably, *nosZ* exists in two phylogenetically distinct clades (*nosZ1* and *nosZ2*) (Jones et al., 2013). In the absence of direct N_2O flux measurements, the ratio of nos to nir gene abundance provides a practical proxy for the soil's potential to mitigate N_2O emissions (Bian et al., 2017; Kandeler et al., 2006).

Given this context, the primary objectives of this study are: (i) To comparatively assess the medium-term (six-year) effects of two contrasting soil management strategies on fast-responding soil quality indicators within Mediterranean greenhouse environments, and (ii) To evaluate the impact of these management practices on the soil denitrification process by analyzing the ratio between N_2O-producing (nir genes) and N_2O-consuming (nos genes).

MATERIALS AND METHODS

Experimental Design

The study was carried out at Palmerillas Experimental Station (Cajamar Foundation), located in the province of Almería (geographical coordinates: 36° 48′N, 2° 3′W; elevation: 155 m). Two adjacent greenhouses with similar soils and comparable crop histories were selected (**Figure 1**). Further baseline soil parameters are available in **Supplementary Material A**.

In each of these greenhouses, two different fertilization systems were established (**Supplementary Material B**). The greenhouse under conventional management (C) received inorganic fertilization applied through fertigation. In contrast, the greenhouse with ecological intensification practices (EI) was fertilized by incorporating crop residues from the previous year (*Solanum melongena* L.), which were mixed with organic amendments (manure). The detailed description of the management systems, as well as the soil total organic carbon (TOC) and total nitrogen (TN) contents of the amendments used (crop residues and manure), can be found in Hernández Maqueda et al. (2024).

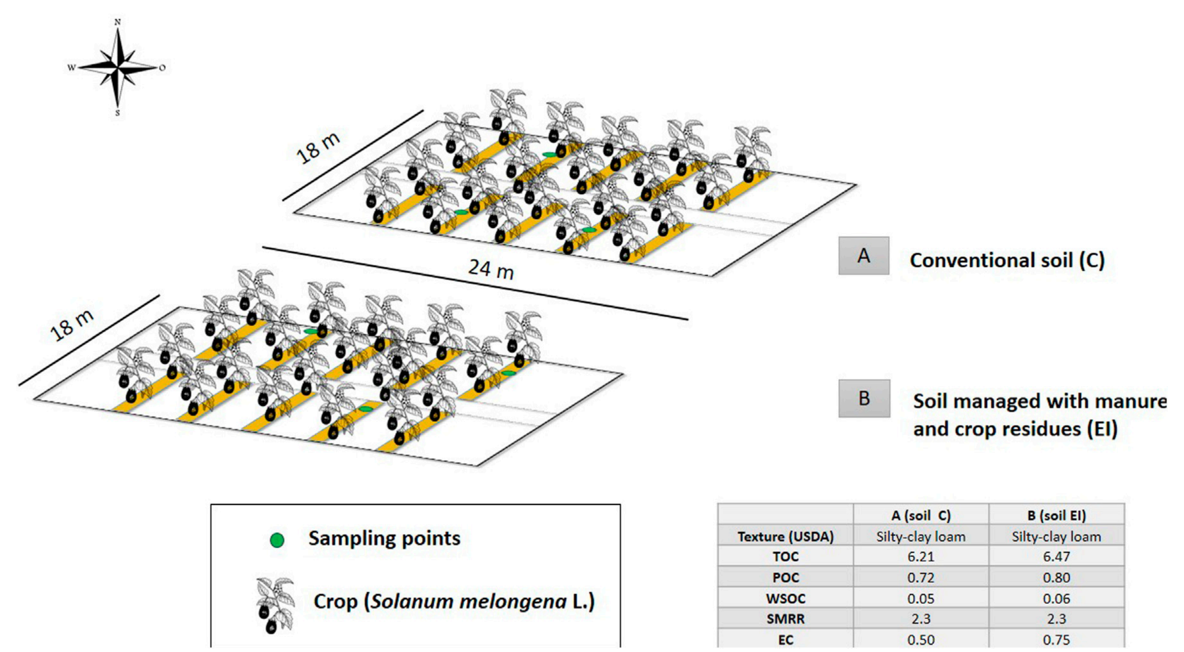
Soil Sampling

Sampling was carried out after 6 years of incorporating organic amendments in the greenhouse under ecological intensification management (I.E.,). The crop at the time of sampling was eggplant (*Solanum melongena*).

The soil sampling was carried out at the end of the growing season, once retired the crop (May 2021). In each greenhouse three drip lines were chosen randomly and, in each of them, a dripper was chosen randomly too. Soil samples were taken at 0–15 cm below the drip line. Once taken, nine soil samples per greenhouse (3 replicates x 3 positions) were stored field-moist in sealed polythene bags at 4 °C until required. A sample aliquot was stored in polythene containers at −80 °C for DNA extraction and molecular biology analysis.

Soil Analyses

Field moist samples were disaggregated and sieved through a 2 mm screen.



	A (soil C)	B (soil EI)
Texture (USDA)	Silty-clay loam	Silty-clay loam
TOC	6.21	6.47
POC	0.72	0.80
WSOC	0.05	0.06
SMRR	2.3	2.3
EC	0.50	0.75

FIGURE 1 | Schematic diagram illustrating the experimental design. **(A)**-Conventional Soil (C) and **(B)**- Soil Managed with Manure and Crop Residues (EI). The table in the lower right corner lists key baseline parameters: TOC (Total Organic Carbon, g kg^{-1}), POC (Particulate Organic Carbon, g kg^{-1}), WSOC (Water Soluble Organic Carbon, g kg^{-1}), SMRR (Soil Microbial Respiration Rate, mg CO_2-C kg^{-1}), and EC (Electrical Conductivity, dS m^{-1}).

Total organic carbon was measured on fine ground soil by wet oxidation method according to Mingorance et al. (2007). Water soluble organic carbon (WSOC) was measured in a 1:2 soil:water suspension (Scaglia and Adani, 2009). Total Nitrogen (TN) was measured using an Elementar Rapid N exceed elemental analyzer. Soil ammonium (NH_4^+) and nitrate (NO_3^-) contents were extracted with 2 mol L^{-1} KCl according to Mulvaney (1996). The analysis of NH_4^+ and NO_3^-were carried out within 3 days after sampling. Electrical conductivity (EC, dSm^{-1}) was measured in a 1:5 soil: water suspension (v/v), with a Crison 522 conductivity meter (UNE 77308:2001), and pH was measured in a 1:5 soil:water suspension (v/v) with a Crison basic 20 pH-meter (UNE-ISO 10390:2012). Dissolved cations were measured in 1:5 soil water suspension (v/v): Ca^{++} and Mg^{++} by atomic absorption spectroscopy. $Na^+$and $K^+$by flame photometry. Finally, the percentage of resistant soil aggregates (RA) was calculated using a Wet Sieving Apparatus (Eijkelkamp).

Nucleic Acid Isolation and Quantitative PCR

DNA was extracted in duplicate from 500 mg of each individual soil sample employing the PowerSoil™DNA Extraction Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentration and purity were estimated spectroscopically using Nanodrop 2000™ Spectrophotometer (Thermo Fisher, Wilmington, Delaware USA). The quantification of bacterial and fungi abundance was performed by Real-Time Quantitative Polimerase Chain Reaction (qPCR) using a 7900HT Fast Real Time PCR System (Applied Biosystems, Foster City, California USA.). PCR reactions were performed in triplicate with a total volume of 20 µL containing 5 µL × iTaq™ universal SYBR® Green supermix (BioRad, Los Angeles, California USA), 2 µL of each primer (10 µM) and 10 ng of template DNA. PCR conditions were, an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Specific primers from conserved regions of the bacteria 16S rRNA gene (Steven et al., 2014) and fungi ITS1-5.8S (Delgado-Delgado-Baquerizo et al., 2016) were used in order to perform the quantitative PCR analysis. Results were expressed as number of copies of bacterial 16S rRNA genes or ITS, per Gram of soil. The same protocol was followed to obtain the abundance of the genes involved in the denitrification pathway (*nirK*, *nirS*, *nosZ1* and *nosZ2*). The primers employed, and the specific real-time PCR conditions for this genes are described by Hernández Maqueda et al. (2024), Hernández Maqueda et al. (2025). Detailed qPCR conditions are described as **Supplementary Material C,D**.

Statistical Analyses

Statistical analyses were performed using the open-source software R version 4.5.1 (R Core Team, 2022). The illustrations were generated with the *ggplot2* package. A linear mixed model (LMM) was employed to examine the influence of management on both the physical, chemical and biological variables included in the study by means of the packages *lme4* and *lmerTest*. To test the assumptions of parametric statistical analyses, all variables were transformed to a natural logarithm, when necessary, to achieve normality and homogeneity of variance, and then examined using the Shapiro-Wilk (S-W) and Bartlett's tests, respectively. The model, following the "REML" specification, utilized residual (restricted) maximum

TABLE 1 | Least square means of the physicochemical variables analyzed 6 years after the implementation of the EI package, comparing the EI and Control (C) treatments, along with the significance of their effects based on the LMM.

Soil properties	EI	C	SED	p	σ_b^2	BIC
TOC (g kg^{-1})	31.6 (4.78)	12.2 (4.25)	2.087	<**0.001**	0.8647	109.639
WSOC (g kg^{-1})	0.68 (0.08)	0.23 (0.02)	0.2756	<**0.001**	0.3698	–27.93151
TN (g kg^{-1})	3.62 (0,67)	2.37 (0.21)	0.2348	<**0.001**	<0.0001	39.06161
Rate C to N	9.1 (2.38)	5.98 (1.97)	1.020	**0.016**	0.0969	86.39749
NH_4-N (mg kg^{-1})	28.6 (2.22)	30.1 (5.05)	1.356	0.283	6.941	103.0441
NO_3^- (mg kg^{-1})	11.0 (4.96)	5.59 (1.83)	1.695	**0.015**	1.031	103.4974
pH	7.9 (0.08)	8.6 (0.14)	0.0333	<**0.001**	0.0082	–11.78364
EC (ds m^{-1})	4.52 (2.63)	0.61 (0.15)	0.8584	**0.0018**	0.1749	81.34386
Ca^{2+} (g kg^{-1})	8.96 (3.69)	13.5 (3.44)	1.552	**0.019**	1.911	101.9054
Mg^{2+} (g kg^{-1})	7.33 (2.84)	4.18 (1.24)	0.8926	**0.0077**	1.241	85.99882
Na^+ (g kg^{-1})	10.6 (7.51)	2.78 (0.87)	2.359	**0.01**	3.559	114.8969
K^+ (g kg^{-1})	3.93 (1.39)	1.22 (0.44)	0.4571	<**0.001**	0.1314	62.34999
WSRA (%)	33.2 (10.4)	21.3 (7.73)	3.358	**0.0076**	33.37	130.9084
Bacteria (log10 copies gr^{-1})	1.65 (0.98)	1.33 (0.68)	0.0024	0.300	<0.001	–101.5627
Fungi	2.11 (1.98)	0.27 (0.18)	0.0041	**0.0005**	6.857	–89.74834
Rate fungi/Bacteria	1.34 (1.25)	0.41 (0.56)	0.4562	0.058	0.000	60.3143
nirK	6.86 (6.55)	1.60 (1.74)	0.0095	0.1742	<0.0001	–60.70995
nirS	1.04 (0.94)	0.9 (0.9)	0.0154	0.972	<0.0001	–47.69097
nosZ1	7.5 (8.17)	2.37 (3.24)	0.0057	**0.004**	0.0002	–68.75209
nosZ2	1.18 (0.84)	0.45 (0.42)	0.0048	**0.001**	<0.0001	–82.42645
Rate *nosZ/nirS*	2.26 (1.53)	3.84 (4.45)	4.128	0.151	26.83	135.0411

TOC, Total Organic Carbon; WSOC, Water Soluble Organic Carbon; TN, Total Nitrogen; EC, Electrical Conductivity. σ2b, Intercept Variance (random effect); SRA, Soil Resistant Aggregates; Abundances of Bacteria, Fungi, nirK, nirS, nosZ1 *and* nosZ2 *are presented as* log_{10} *copies* gr^{-1} *dry soil; Standard deviations are presented in parentheses. SED, standard error of the differences of means; Bold values indicate significant differences in soil properties* ($p < 0.05$) *according to the LMM; BIC, Bayesian information criterion.*

likelihood estimation. The fixed effects included EI application versus control, and dripline was considered a random effect (RE), capturing random deviations for each sample from the overall fixed intercept. The goodness of fit of the best model was assessed using the Bayesian Information Criterion (BIC). Correlations were calculated with the package *Hmisc*. The relationships between the DNA abundances and the physicochemicals variables were assessed by meaning of a Spearman's Rho nonparametric test. Significant correlations ($p < 0.05$) were adjusted using the False Discovery Rate (FDR). Graphics were performed with the library *corrplot*.

RESULTS

Effects of EI Management on Soil Properties

According to the linear mixed model (LMM), statistically significant differences were detected between the two management systems in relation to the soil's physical and chemical properties, with the exception of the ammonium ion (NH_4^+). With regard to the biological properties analyzed, a substantial increase was observed in fungal DNA abundance, as well as in the copy numbers of the *nosZ1* and *nosZ2* genes involved in the reduction of N_2O to N_2 (**Table 1**).

When analyzing the proportional increase of each variable in soil managed with EI compared to conventionally managed soil (**Figure 2**), we found that the physicochemical parameter with the greatest increase is electrical conductivity (EC), which is 7.4 fold higher than under conventional management.

The analyzed organic carbon fractions (TOC and WSOC) more than doubled under EI management. Total nitrogen (TN) increased 1.5-fold, while ammonium (NH_4^+) remained at similar levels, and nitrate (NO_3^-) nearly doubled compared with conventional management. Consequently, the C to N ratio increased 1.5-fold. Among the soluble cations, sodium (Na^+) increased four-fold, potassium (K^+) three-fold, and magnesium (Mg^{2+}) 1.7-fold, whereas calcium (Ca^{2+}) decreased by 34%. Additionally, soil pH declined by approximately 9%, averaging a decrease of 0.7 units, and the percentage of soil resistant aggregates (SRA), an indicator of soil structural stability, increased roughly 1.5-fold.

Bacterial abundance measured by the 16S gene remained similar in both treatments (1.65 vs. 1.33 log_{10} copies g^{-1} dry soil). However, a substantial increase was observed in the number of ITS2 gene copies used to detect fungal abundance (2.11 vs. 0.27 log_{10} copies g^{-1} dry soil). Consequently, the Fungi/Bacteria ratio also increased threefold.

All genes analyzed to evaluate the denitrification process increased in EI-managed soil, with the increase being statistically significant for both variants of the nitrous oxide reductase genes (*nosZ*1 and *nosZ*2).

Interaction Between DNA Abundance and Soil Physicochemical Properties

The correlation analysis based on Spearman's Rho between the abundances of various genes and the different soil parameters revealed the following main findings (**Figure 3**):

i. TOC correlated positively (Rho = 0.700, p = 0.008) with the abundance of ITS2*;*

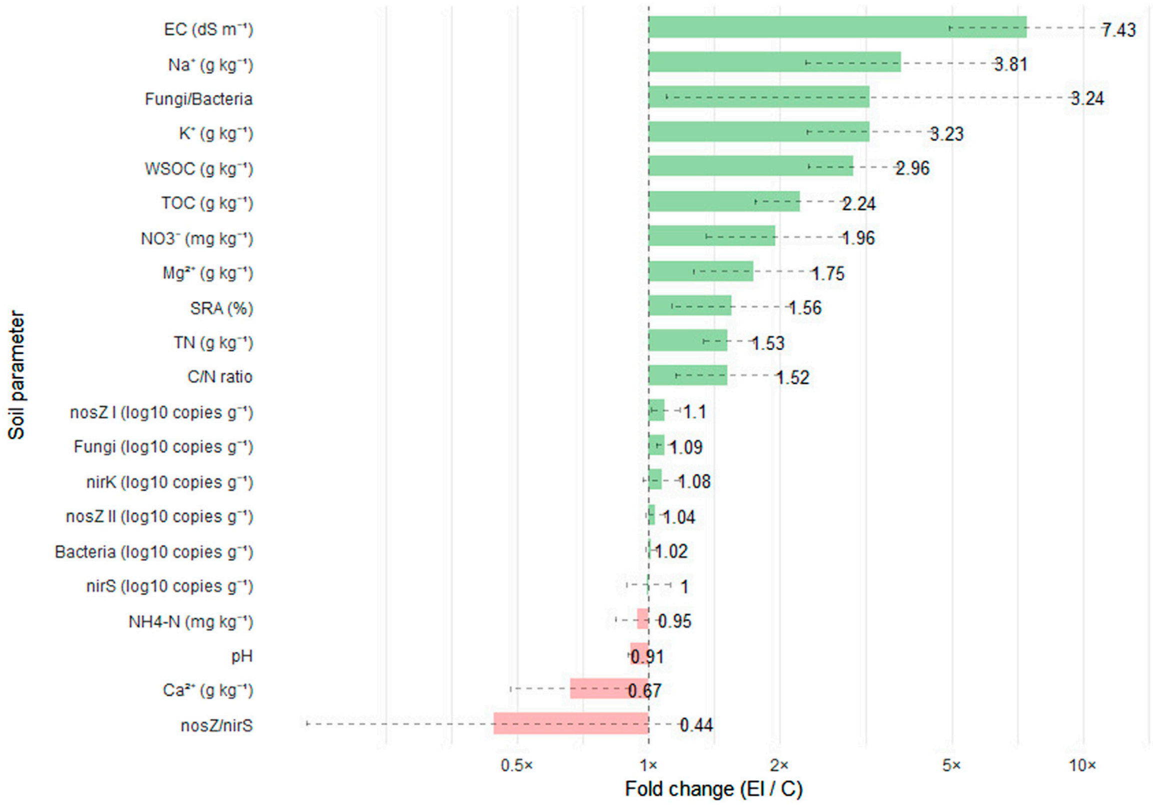


FIGURE 2 | Log-scale representation of the relative (x-fold) increase in the different soil parameters under ecological intensification (EI) management compared with conventional management (C). The 95% confidence intervals are shown as dashed lines.

ii. The C to N ratio showed positive correlations with all genes, although statistical significance was only observed for ITS2 (Rho = 0.626, p = 0.024);
iii. Ammonium ion (NH_4^+) was positively correlated with *nirS* genes (Rho = 0.589, p = 0.04);
iv. Negative correlation between pH and ITS2 (Rho = - 0.604, p = 0.032), *nirK* (Rho = −0.587, 0.004) and *nosZ1* (Rho = −0.677, p = 0.001) gene copy number;
v. The DNA copy numbers of *nirK, nirS, nosZ1*, and *nosZ2* were positively correlated with each other (p < 0.05);

DISCUSSION

Ecological intensification (EI) management resulted in higher values for most of the quality indicators related to organic matter and biodiversity. Notably, total organic carbon (TOC) under EI management nearly doubled compared to conventional management. This increase exceeds the values typically reported in the literature (Bongiorno et al., 2019; Geraei et al., 2016; Haynes, 2005). However, despite this significant improvement, TOC levels remain below the optimal thresholds recommended for greenhouse horticultural systems (Fernández et al., 2014).

The incorporation of crop residues and compost as organic amendments contributed to also increased water-soluble organic carbon (WSOC), consistent with findings by Bongiorno et al. (2019). WSOC exhibited the highest proportional increase among carbon-related indicators in the EI treatment, though a substantial increase was also observed under conventional (C) management. This suggests that WSOC levels may be influenced by factors beyond the scope of this study's management practices (Chantigny, 2003).

Although some studies have demonstrated a strong link between WSOC and microbial communities—where WSOC serves both as a byproduct of microbial decomposition and an accessible energy source for microbes (Guigue et al., 2015)—our findings revealed a weak correlation between WSOC and biodiversity-related indicators. This disconnect suggests that WSOC alone may not reliably reflect shifts in microbial diversity or abundance.

Microbial Community Shifts and Carbon Dynamics

Real-time PCR analyses showed substantial increases in biological indicators following EI management, indicating an overall enhancement of microbial presence. Both fungi and

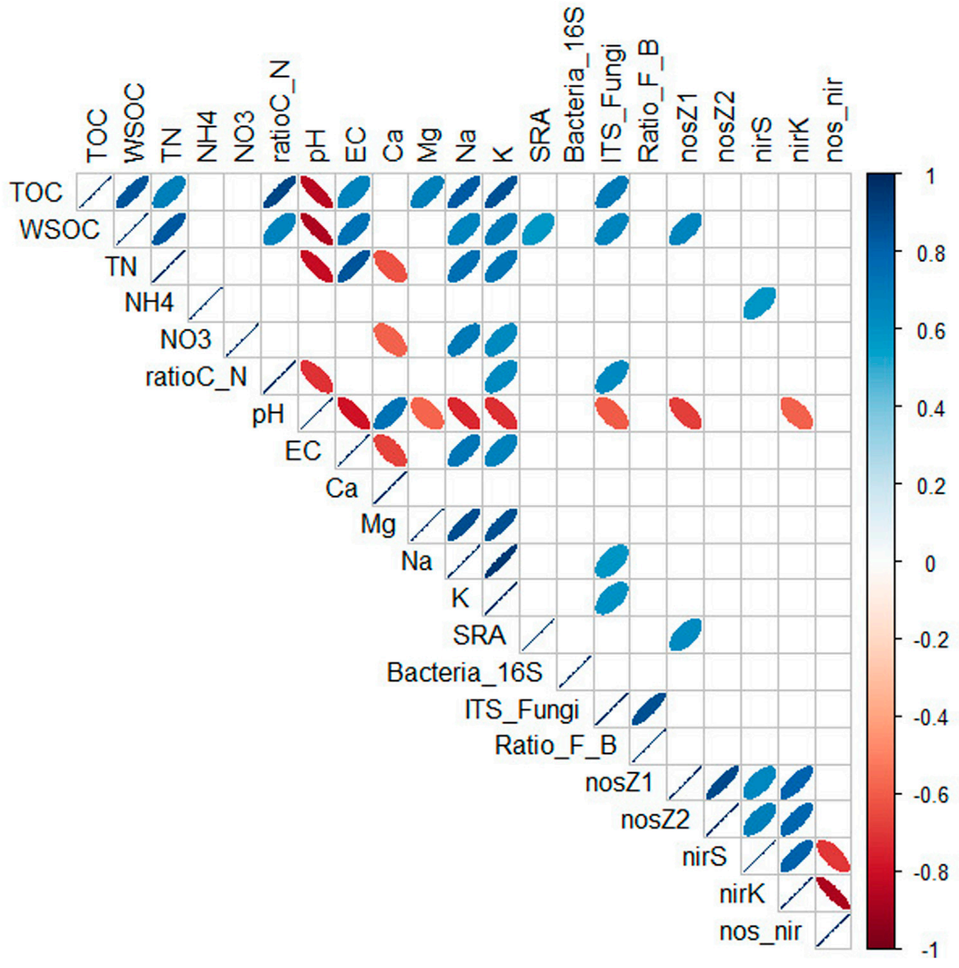


FIGURE 3 | Correlation matrix of the analyzed parameters based on Spearman's Rho nonparametric test. Significant correlations ($p < 0.05$), adjusted using the False Discovery Rate (FDR), are highlighted with ellipses.

bacteria are key decomposers in soil, though they differ in the types of carbon substrates they utilize (Lauber et al., 2008). In this study, both management systems received equivalent amounts of nitrogen, though in different forms: the conventional system received 100% as mineral nitrogen, while only 40% of the nitrogen applied in the EI system was in soluble form.

Despite this, bacterial abundance did not significantly differ between treatments. In contrast, fungal abundance was markedly higher under EI management. This is likely due to the incorporation of lignified eggplant crop residues and mature compost, which favor fungal proliferation (Ciaccia et al., 2019; Denef et al., 2001). Consequently, the fungal-to-bacterial ratio increased under EI, consistent with observations by Malik et al. (2016). Intensive fertilization, as commonly practiced in conventional systems, is known to reduce the fungal to bacterial ratio by favoring bacterial dominance (de Vries et al., 2006; Parfitt et al., 2012). Additionally, the Fungal to Bacteria ratio is strongly influenced by the carbon-to-nitrogen (C: N) ratio (Xiao et al., 2022; Waring et al., 2013). In our study, the C to N ratio rose from 5.98 to 9.1 under EI management, potentially contributing to the higher fungal abundance observed.

Impacts on Denitrification Genes

Under EI management, we observed increases in the copy numbers of key denitrification genes, particularly *nosZ1* and *nosZ2* (nitrous oxide reductases). This finding aligns with previous studies indicating that organic nitrogen inputs promote denitrifier communities (You et al., 2022; Ouyang et al., 2018). Nevertheless, gene abundance is not solely dependent on fertilization type. Recent research has highlighted the importance of pH and TOC as major drivers of microbial abundance and diversity (Dong et al., 2023; Xiao et al., 2021). Other factors such as crop developmental stage, soil moisture, and salinity also influence denitrification genes abundance, particularly in Mediterranean greenhouse systems (Hernández Maqueda et al., 2024; Hernández Maqueda et al., 2025).

A notable finding in both treatments was that the ratio of N_2O-consuming (nos genes) to N_2O-producing (nir genes) organisms was greater than 2, suggesting a potential to minimize NO_3^- accumulation and N_2O formation. However, caution is warranted when interpreting this indicator, as denitrification is only one of several microbial pathways that contribute to N_2O emissions (Dong et al., 2023).

The observed microbial shifts under EI management suggest that fungi play an increasingly central role in carbon cycling and potentially in denitrification processes. While a direct correlation between fungal abundance (measured via ITS gene copies) and denitrification genes was not detected in this study, the concurrent increases in ITS, *nosZ1*, and *nosZ2* gene copy numbers under EI management point to a possible association. This highlights a need for further research into the functional roles of fungal communities in denitrification. Recent work has emphasized that the role of fungi in nitrogen cycling has been underestimated (Aldossari and Ishii, 2021), particularly in systems where fungi dominate decomposition processes, such as those amended with compost and crop residues.

Salinization Risk as a Trade-Off

Despite the observed improvements in soil organic matter, microbial abundances, and structural stability (as indicated by increased soil resistant aggregates), EI management was also associated with a remarkable rise in soil electrical conductivity (EC). This suggests a potential salinization risk. The use of eggplant residues, which are high in mineral salts, and compost derived from greenhouse crop residues contributed significantly to the increase in soil salinity. In this case study, Mg^{2+}, Na^+, and K^+ increased considerably, whereas Ca^{2+} decreased. A possible explanation for the reduction in Ca^{2+} is that the presence of organic acids derived from the incorporation of crop residues may have promoted the leaching of this cation, as observed in the study by Jones et al. (2012).

To mitigate this risk, it is advisable to reconsider the types of organic amendments used. Specifically, avoiding high-salt biomass inputs and exploring alternative combinations of fresh and mature organic matter may help maintain soil quality without increasing salinity. This approach would also reduce the need for salt leaching, a practice that may have adverse effects on groundwater quality.

CONCLUSION

Considering the limitations of this study, which arise from its exploratory, case-study design, several general conclusions can be drawn. Changes in soil conditions resulting from tillage and the incorporation of crop residues and compost under ecological intensification have led to marked increases in total organic matter and water-soluble organic carbon (WSOC). These amendments also improved overall biological indicators, particularly by significantly enhancing the relative abundance of fungi compared to bacteria. However, a potential drawback of the selected organic materials is the associated increase in soil electrical conductivity, indicating an elevated risk of salinization within the greenhouse system.

With the exception of bacterial abundance, all evaluated soil quality indicators responded sufficiently over the six-year period to differentiate between the two management strategies. This responsiveness underscores their suitability for monitoring management effects, particularly in greenhouse production systems where rapid feedback is critical for decision-making.

Furthermore, the abundance of denitrification-related genes increased under ecological intensification, with significant effects observed for the nitrous oxide reductase genes (*nosZ1* and *nosZ2*), suggesting a potential positive impact on nitrogen cycling. To further elucidate the relationship between microbial communities and N_2O emissions, future studies should incorporate high-throughput sequencing approaches alongside more detailed monitoring of the denitrification process, including measurements of leachates and N_2O emissions. Such approaches will provide a more comprehensive understanding of bacterial diversity and community structure associated with denitrification.

DATA AVAILABILITY STATEMENT

Data set from soil samples and the statistical analyses performed can be downloaded from the following repository: https://doi.org/10.5281/zenodo.17750765. Additional data or additional information is available upon request from the corresponding author.

AUTHOR CONTRIBUTIONS

FM conducted the physicochemical analyses, RH conducted the biological analyses, RH wrote the initial manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/sjss.2025.15184/full#supplementary-material

REFERENCES

Aldossari, N., and Ishii, S. (2021). Fungal Denitrification Revisited-Recent Advancements and Future Opportunities. *Soil Biol. biochem.* 157, 108250. doi:10.1016/j.soilbio.2021.108250

Bai, Z., Caspari, T., Ruiperez, M., Batjes, N. H., Mäder, P., Bünemann, E. K., et al. (2018). Effects of Agricultural Management Practices on Soil Quality: A Review of Long-Term Experiments for Europe and China. *Agric. Ecosyst. Environ.* 265, 1–7. doi:10.1016/j.agee.2018.05.028

Bender, S. F., Wagg, C., and van der Heijden, M. G. A. (2016). An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. *Trends Ecol. Evol.* 31 (6), 440–452. doi:10.1016/j.tree.2016.02.016

Bian, R., Sun, Y., Li, W., Ma, Q., and Chai, X. (2017). Co-Composting of Municipal Solid Waste Mixed with Matured Sewage Sludge: The Relationship Between N_2O Emissions and Denitrifying Gene Abundance. *Chemosphere* 189, 581–589. doi:10.1016/j.chemosphere.2017.09.070

Bonachela, S., Fernández, M. D., Cabrera, F. J., and Granados, M. R. (2018). Soil Spatio-Temporal Distribution of Water, Salts and Nutrients in Greenhouse, Drip-Irrigated Tomato Crops Using Lysimetry and Dielectric Methods. *Agric. Water Manag.* 203, 151–161. doi:10.1016/j.agwat.2018.03.009

Bongiorno, G., Bünemann, E. K., Oguejiofor, C. U., Meier, J., Gort, G., Comans, R., et al. (2019). Sensitivity of Labile Carbon Fractions to Tillage and Organic Matter Management and Their Potential as Comprehensive Soil Quality Indicators Across Pedoclimatic Conditions in Europe. *Ecol. Indic.* 99, 38–50. doi:10.1016/j.ecolind.2018.12.008

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., et al. (2018). Soil Quality – A Critical Review. *Soil Biol. biochem.* 120, 105–125. doi:10.1016/j.soilbio.2018.01.030

Cabrera, F. J., Bonachela, S., Fernández, M. D., Granados, M. R., and López, J. C. (2016). Lysimetry Methods for Monitoring Soil Solution Electrical Conductivity and Nutrient Concentration in Greenhouse Tomato Crops. *Agric. Water Manag.* 178, 171–179. doi:10.1016/j.agwat.2016.09.024

Chantigny, M. H. (2003). Dissolved and Water-Extractable Organic Matter in Soils: A Review on the Influence of Land Use and Management Practices. *Geoderma* 113, 357–380. doi:10.1016/S0016-7061(02)00370-1

Ciaccia, C., La Torre, A., Ferlito, F., Testani, E., Battaglia, V., Salvati, L., et al. (2019). Agroecological Practices and Agrobiodiversity: A Case Study on Organic Orange in Southern Italy. *Agronomy* 9, 85. doi:10.3390/agronomy9020085

Cuevas, J., Daliakopoulos, I. N., del Moral, F., Hueso, J. J., and Tsanis, I. K. (2019). A Review of Soil-Improving Cropping Systems for Soil Salinization. *Agronomy* 9, 295. doi:10.3390/agronomy9060295

De Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., and Bloem, J. (2006). Fungal/Bacterial Ratios in Grasslands with Contrasting Nitrogen Management. *Soil Biol. biochem.* 38, 2092–2103. doi:10.1016/j.soilbio.2006.01.008

Del Moral, F., González, V., Simón, M., García, I., Sánchez, J. A., and de Haro, S. (2012). Soil Properties After 10 Years of Organic Versus Conventional Management in Two Greenhouses in Almeria (SE Spain). *Arch. Agron. Soil Sci.* 58, S226–S231. doi:10.1080/03650340.2012.698730

Delgado-Baquerizo, M., Maestre, F. T., Eldridge, D. J., Bowker, M. A., Ochoa, V., Gozalo, B., et al. (2016). Biocrust-Forming Mosses Mitigate the Negative Impacts of Increasing Aridity on Ecosystem Multifunctionality in Drylands. *New Phytol.* 209, 1540–1552. doi:10.1111/nph.13688

Denef, K., Six, J., Bossuyt, H., Frey, S. D., Elliott, E. T., Merckx, R., et al. (2001). Influence of Dry - Wet Cycles on the Interrelationship Between Aggregate, Particulate Organic Matter, and Microbial Community Dynamics. *Soil Tillage Res.* 33, 1599–1611. doi:10.1016/S0038-0717(01)00076-1

Dong, Y., Xu, X., Zhang, J., Jiao, Y., Wang, B., Wang, C., et al. (2023). Contributions of Ammonia-Oxidizing Archaea and Bacteria to Nitrous Oxide Production in Intensive Greenhouse Vegetable Fields. *Agronomy* 13, 2420. doi:10.3390/agronomy13092420

FAO & ITPS (2015). *World ' S Soil Resources, ISBN 9789251090046.*

Fernández, M. M., Aguilar, M. I., Carrique, J. R., Tortosa, J., García, C., López, M., et al. (2014). "*Suelo y Medio Ambiente En Invernaderos*; Junta de Andalucía," in *Secretaría General Técnica, Servicio de Publicaciones y Divulgación: Almería (Spain)*. Editor P. y. D. R. Consejería de Agricultura 5th ed.

Gallardo, M., Giménez, C., Martínez-Gaitán, C., Stöckle, C. O., Thompson, R. B., and Granados, M. R. (2011). Evaluation of the VegSyst Model with Muskmelon to Simulate Crop Growth, Nitrogen Uptake and Evapotranspiration. *Agric. Water Manag.* 101, 107–117. doi:10.1016/j.agwat.2011.09.008

Geraei, D. S., Hojati, S., Landi, A., and Cano, A. F. (2016). Total and Labile Forms of Soil Organic Carbon as Affected by Land Use Change in Southwestern Iran. *Geoderma Reg.* 7, 29–37. doi:10.1016/j.geodrs.2016.01.001

Ghiglieno, I., Simonetto, A., Donna, P., Tonni, M., Valenti, L., Bedussi, F., et al. (2019). Soil Biological Quality Assessment to Improve Decision Support in the Wine Sector. *Agronomy* 9, 593. doi:10.3390/agronomy9100593

Granados, M. R., Thompson, R. B., Fernández, M. D., Martínez-Gaitán, C., and Gallardo, M. (2013). Prescriptive-Corrective Nitrogen and Irrigation Management of Fertigated and Drip-Irrigated Vegetable Crops Using Modeling and Monitoring Approaches. *Agric. Water Manag.* 119, 121–134. doi:10.1016/j.agwat.2012.12.014

Guigue, J., Lévêque, J., Mathieu, O., Schmitt-Kopplin, P., Lucio, M., Arrouays, D., et al. (2015). Water-Extractable Organic Matter Linked to Soil Physico-Chemistry and Microbiology at the Regional Scale. *Soil Biol. biochem.* 84, 158–167. doi:10.1016/j.soilbio.2015.02.016

Haynes, R. J. (2005). Labile Organic Matter Fractions as Central Components of the Quality of Agricultural Soils: An Overview. *Adv. Agron.* 85, 221–268. doi:10.1016/S0065-2113(04)85005-3

Hernández Maqueda, R., Ballesteros, I., Meca, D., Linacero, R., and del Moral, F. (2024). Ecological Intensification Strategies Increase Abundance of Denitrifying Functional Genes in a Greenhouse Agricultural-Soil. *Appl. Soil Ecol.* 199, 105415. doi:10.1016/j.apsoil.2024.105415

Hernández Maqueda, R., Ballesteros, I., Aguirre, A. J., Meca, D., Linacero, R., and del Moral, F. (2025). Influence of green manuring on soil properties, abundance and expression of key denitrification genes in a greenhouse Anthrosol. *Appl. Soil Ecol.* 212, 106216. doi:10.1016/j.apsoil.2025.106216

Hill, G. T., Mitkowski, N. A., Aldrich-Wolfe, L., Emele, L. R., Jurkonie, D. D., Ficke, A., et al. (2000). Methods for Assessing the Composition and Diversity of Soil Microbial Communities. *Appl. Soil Ecol.* 15, 25–36. doi:10.1016/S0929-1393(00)00069-X

Jones, B. E., Haynes, R. J., and Phillips, I. R. (2012). Addition of an Organic Amendment and/or Residue Mud to Bauxite Residue Sand in Order to Improve Its Properties as a Growth Medium. *J. Environ. Manage.* 95 (1), 29–38. doi:10.1016/j.jenvman.2011.09.014

Jones, C., Graf, D., Bru, D., Philippot, L., and Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *ISME J.* 7, 417–426. doi:10.1038/ismej.2012.125

Juhos, K., Czigány, S., Madarász, B., and Ladányi, M. (2019). Interpretation of Soil Quality Indicators for Land Suitability Assessment – A Multivariate Approach for Central European Arable Soils. *Ecol. Indic.* 99, 261–272. doi:10.1016/j.ecolind.2018.11.063

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., and Philippot, L. (2006). Abundance of narG, nirS, nirK, and nosZ Genes of Denitrifying Bacteria During Primary Successions of a Glacier Foreland. *Appl. Environ. Microbiol.* 72 (9), 5957–5962. doi:10.1128/AEM.00439-06

Kuypers, M. M. M., Marchant, H. K., and Kartal, B. (2018). The Microbial Nitrogen-Cycling Network. *Nat. Rev. Microbiol.* 16, 263–276. doi:10.1038/nrmicro.2018.9

Lauber, C. L., Strickland, M. S., Bradford, M. A., and Fierer, N. (2008). The Influence of Soil Properties on the Structure of Bacterial and Fungal Communities Across Land-Use Types. *Soil Biol. biochem.* 40, 2407–2415. doi:10.1016/j.soilbio.2008.05.021

Li, P., Shi, K., Wang, Y., Kong, D., Liu, T., Jiao, J., et al. (2019). Soil Quality Assessment of Wheat-Maize Cropping System with Different Productivities in China: Establishing a Minimum Data Set. *Soil Tillage Res.* 190, 31–40. doi:10.1016/j.still.2019.02.019

Lin, Z.-Q., and Bañuelos, G. S. (2015). “Soil Salination Indicators,” in *Environmental Indicators*. Editors R. H. Armon and O. Hänninen (Dordrecht: Springer Netherlands), 319–330.

Malik, A. A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P. G. M., et al. (2016). Soil Fungal: Bacterial Ratios Are Linked to Altered Carbon Cycling. *Front. Microbiol.* 7, 1–11. doi:10.3389/fmicb.2016.01247

Mingorance, M. D., Barahona, E., and Fernández-Gálvez, J. (2007). Guidelines for Improving Organic Carbon Recovery by the Wet Oxidation Method. *Chemosphere* 68, 409–413. doi:10.1016/j.chemosphere.2007.01.021

Mulvaney, R. L. (1996). “Nitrogen-Inorganic Forms,” in *Methods of Soil Analysis. Part 3. Chemical Methods, SSSA Book Series*. Editors D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loepert, P. N. Soltanpour, and M. A. Tabatabai (Madison, Wisconsin, USA: Soil Science Society of America, Inc. American Society of Agronomy, Inc.), 1123–1184.

Norris, C. E., and Congreves, K. A. (2018). Alternative Management Practices Improve Soil Health Indices in Intensive Vegetable Cropping Systems: A Review. *Front. Environ. Sci.* 6, 50. doi:10.3389/fenvs.2018.00050

Obade, V. de P. (2019). Integrating Management Information with Soil Quality Dynamics to Monitor Agricultural Productivity. *Sci. Total Environ.* 651, 2036–2043. doi:10.1016/j.scitotenv.2018.10.106

Obade, V. D. P., and Lal, R. (2016). Towards a Standard Technique for Soil Quality Assessment. *Geoderma* 265, 96–102. doi:10.1016/j.geoderma.2015.11.023

Oenema, O., Heinen, M., Rietra, R., and Hessel, R. (2017). in *A Review of Soil-Improving Cropping Systems*. Editors O. Oenema, M. Heinnen, R. Rene, and R. Hessel

Ouyang, Y., Evans, S. E., Friesen, M. L., and Tiemann, L. K. (2018). Effect of Nitrogen Fertilization on the Abundance of Nitrogen Cycling Genes in Agricultural Soils: A Meta-Analysis of Field Studies. *Soil Biol. biochem.* 127, 71–78. doi:10.1016/j.soilbio.2018.08.024

Parfitt, R. L., Couper, J., Parkinson, R., Schon, N. L., and Stevenson, B. A. (2012). Effect of Nitrogen Fertilizer on Nitrogen Pools and Soil Communities Under Grazed Pastures. *New zeal. J. Agric. Res.* 55, 217–233. doi:10.1080/00288233.2012.670118

R Core Team (2022). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R foundation for Statistical Computing. Available online at: https://www.R-project.org/.

Salinas, J., Meca, D., and del Moral, F. (2020). Short-Term Effects of Changing Soil Management Practices on Soil Quality Indicators and Crop Yields in Greenhouses. *Agronomy* 10 (4), 582. doi:10.3390/agronomy10040582

Scaglia, B., and Adani, F. (2009). Biodegradability of Soil Water Soluble Organic Carbon Extracted from Seven Different Soils. *J. Environ. Sci.* 21, 641–646. doi:10.1016/S1001-0742(08)62319-0

Seitzinger, S. P., and Phillips, L. (2017). Nitrogen Stewardship in the Anthropocene. *Science* 357 (6349), 350–351. doi:10.1126/science.aao0812

Steven, B., Gallegos-Graves, L. V., Yeager, C., Belnap, J., and Kuske, C. R. (2014). Common and Distinguishing Features of the Bacterial and Fungal Communities in Biological Soil Crusts and Shrub Root Zone Soils. *Soil Biol. biochem.* 69, 302–312. doi:10.1016/j.soilbio.2013.11.008

Urra, J., Alkorta, I., and Garbisu, C. (2019). Potential Benefits and Risks for Soil Health Derived from the Use of Organic Amendments in Agriculture. *Agronomy* 9, 542. doi:10.3390/agronomy9090542

Vereecken, H., Schnepf, A., Hopmans, J. W., Javaux, M., Or, D., Roose, T., et al. (2016). Modeling Soil Processes: Review, Key Challenges, and New Perspectives. *Vadose Zo. J.* 15, 0–57. doi:10.2136/vzj2015.09.0131

Wander, M. M., and Bidart, M. G. (2000). Tillage Practice Influences on the Physical Protection, Bioavailability and Composition of Particulate Organic Matter. *Biol. Fertil. Soils* 32, 360–367. doi:10.1007/s003740000260

Waring, B. G., Averill, C., and Hawkes, C. V. (2013). Differences in Fungal and Bacterial Physiology Alter Soil Carbon and Nitrogen Cycling: Insights from Meta-Analysis and Theoretical Models. *Ecol. Lett.* 16 (7), 887–894. doi:10.1111/ele.12125

Xiao, X., Xie, G., Yang, Z., He, N., Yang, D., and Liu, M. (2021). Variation in Abundance, Diversity, and Composition of *nirK* and *nirS* Containing Denitrifying Bacterial Communities in a Red Paddy Soil as Affected by Combined Organic-Chemical Fertilization. *Appl. Soil Ecol.* 166, 104001. doi:10.1016/j.apsoil.2021.104001

Xiao, D., He, X., Wang, G., Xu, X., Hu, Y., Chen, X., et al. (2022). Network Analysis Reveals Bacterial and Fungal Keystone Taxa Involved in Straw and Soil Organic Matter Mineralization. *Appl. Soil Ecol.* 173, 104395. doi:10.1016/j.apsoil.2022.104395

Yazdanpanah, N., Mahmoodabadi, M., and Cerdà, A. (2016). The Impact of Organic Amendments on Soil Hydrology, Structure and Microbial Respiration in Semiarid Lands. *Geoderma* 266, 58–65. doi:10.1016/j.geoderma.2015.11.032

You, L., Ros, G. H., Chen, Y., Yang, X., Cui, Z., Liu, X., et al. (2022). Global Meta-Analysis of Terrestrial Nitrous Oxide Emissions and Associated Functional Genes Under Nitrogen Addition. *Soil Biol. biochem.* 165, 108523. doi:10.1016/j.soilbio.2021.108523