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Factors Driving Soil Respiration Rate After Different Fertilizer Sources Addition

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Abstract: Soil respiration is a critical process that regulates key ecosystem functions such as climate control, nutrient cycling, and plant productivity. Soil texture, nutrient availability, and microbial communities can all influence soil respiration, yet our understanding of their relative importance remains limited. This study aimed to investigate how different factors—like soil texture, nutrient additions, and microbial communities—contribute to soil respiration and define their specific roles in its variability. Using a microcosm experiment with various fertilizers and two soil types (Navarra, a silty clay soil, and Saponi, a sandy soil), we measured changes in both biotic and abiotic factors. A multiple linear regression analysis revealed that, among other biotic and abiotic factors, soil clay content, soluble nitrogen levels, bacterial abundance, and α-diversity significantly impacted soil respiration, together accounting for over 60% of its total variance. Structural equation modeling indicated that microbial communities made the greatest contribution to respiration at 30.84%, followed by soil texture at 19.63%. Overall, biotic factors were found to play a more significant role in driving soil respiration rates compared to abiotic factors, with edaphic properties having a greater influence than fertilizer additions.

Keywords: soil respiration; bacteria; fungi; soil texture; microbial community

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1. Introduction

Carbon dioxide and other greenhouse gases are major contributors to global temperature rises and climate change [1]. In addition to the large amounts of carbon dioxide released by human activities such as industrial processes, transportation, and energy production, soil microbial respiration also contributes to atmospheric carbon dioxide by utilizing carbon from soil organic matter, releasing an estimated 60 Pg of carbon annually. This contributes to changes in atmospheric greenhouse gas concentrations [2]. Furthermore, research has indicated that soil heterotrophic respiration in many ecosystems has been gradually increasing over the past few decades, amplifying its impact on greenhouse gases [3]. Therefore, studying soil respiration is essential for mitigating greenhouse gas emissions.

Soil respiration governs critical ecosystem functions such as climate regulation, nutrient cycling, and plant productivity [4]. Consequently, there is increasing interest in understanding the factors that influence soil respiration. The key factors include temperature [2,5], precipitation [6,7], vegetation [8], and soil properties [9,10]. Although climate factors (temperature and precipitation) are the primary drivers [11], soil properties often take precedence at a local scale in agricultural systems.

Recently, a meta-analysis of global patterns of soil heterotrophic respiration highlighted the varying roles of climate, soil properties, environment, vegetation, and ecosystem type in driving heterotrophic respiration, but it did not consider microbial communities [11]. Ebrahimi et al. estimated soil respiration across different land uses, including crops, pastures, woodlands, conifers, broadleaf forests, deserts, and salt marshes, demonstrating that soil microbial communities—particularly bacterial communities—play a significant role in soil respiration [12]. A long-term fertilizer input experiment provided new insight that the microbial community can be accounted the major driver in soil respiration [13]. Moreover, soil texture, such as clay content, may not only affect the degradation of soil organic matter [14] but is also considered as a key driver in converting organic matter into soil organic carbon for microbial activity [15,16]. Studies have indicated that soils with finer textures (clay + silt) exhibit a higher carbon retention capacity and stability [17,18]. Considering these factors, it can be inferred that both abiotic factors (like soil texture, total organic carbon, soluble carbon, microbial biomass carbon, and soluble nitrogen) and biotic factors (such as microbial communities, biodiversity, richness, and abundance) may influence soil respiration. However, there remains a lack of in-depth understanding regarding the relative importance of these factors in contributing to soil respiration.

Currently, many studies focus on the effects of fertilizer addition on soil respiration, but the results remain controversial. Some earlier studies observed that fertilizer addition significantly increased soil respiration [19–22], while others observed the opposite trend [23], and some studies reported no effect [24,25]. Given these conflicting findings, we hypothesize that soil respiration is primarily influenced by different fertilizers. The aim of this study is to understand how different factors, such as soil texture, nutrient addition, and microbial communities, affect soil respiration under different forms of fertilizer addition and to explain the contribution of these factors to changes in soil respiration. Through a microcosm experiment, we used two types of soil with a similar total organic carbon and total nitrogen but different textures, adding the same level of nitrogen of fertilizer from different sources. We focused on the contributions of biotic and abiotic factors to soil respiration, employing a multiple linear regression analysis and structural equation modeling to clarify the contribution rates of different factors. The goal is to provide a theoretical basis for green, low-carbon, and sustainable agricultural development.

2. Materials and Methods

2.1. Experimental Design and Sampling

Two different soils were selected for microcosm experiment; the Navarra soil was a silty clay soil, and the Saponi soil was sandy soil (Table 1). Navarra refers to an agricultural area (cereals) in the area of Ferrara (Po Valley); Saponi refers to an area used for horticulture in the area of Rimini (Adriatic sea coast) (Figure 1).

Table 1. Soil characteristics of Navarra and Saponi soil.

Soil	TOC/%	TN/%	TP g/kg	pH	Sand/%	Silt/%	Clay/%
Navarra	0.93	0.15	0.877	7.78	13	71	16
Saponi	1.06	0.11	1.639	7.76	76	16	8

DAP is short for Diammonium phosphate [$(NH_4)_2HPO_4$]; two substrates deriving from the composting process were also added: ACM (Ammendante Compostato Misto, TOC 26.7%; TN 2.7%; TP 4.630 g/kg) is a commercial product derived from the composting

of the organic fraction of municipal solid waste, whereas ACFA (Ammendante Compostato da Fanghi, TOC 27.8%; TN 2.2%; TP 4.228 g/kg) is a compost produced from agro-industrial waste after an anaerobic digestion process.

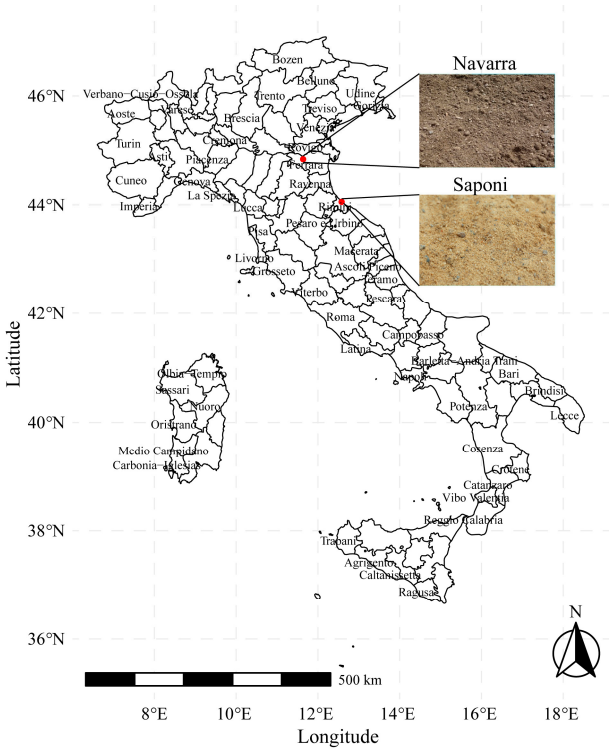

Figure 1. The land use map of the study areas.

Four different conditions were assayed: soil without any amendments (CONC) and soil amended with DAP, ACM, and ACFA to reach a total amount of 200 mg nitrogen/kg dry soil.

All treatments were in triplicate. The soil was sampled at day 0, 7, 14, and 28. The temperature was kept at 24 °C, with twelve hours to the day and night; weight control was performed to keep 50% of water-holding capacity for each sample. Each sampling time took soil samples for tests of both soil chemistry and microbiology. The soil samples were stored for microbiology analysis at −100 °C. Fresh soil underwent examination for humidity and extraction for microbial biomass carbon.

2.2. Edaphic Properties and Soil Respiration Analysis

Total organic carbon was measured with an elemental analyzer (Flash 2000 Thermo-Scientific, Waltham, MA, USA). Soil samples before analysis were pre-treated to eliminate the carbonates. The soil samples, finely ground, were weighed (10–12 mg) in silver capsules placed on a heating plate set at 80 °C, and a few drops of HCl 6 M were added to the soil directly in the capsules until the end of the effervescence. Then the samples were heated until dried, cooled in the desiccator, and analyzed for organic carbon content.

Microbial biomass carbon, soluble carbon and nitrogen: At every sampling time, the microbial biomass carbon (MBC) was measured according to the fumigation–extraction method [26], with slight modifications. An extraction ratio of 1:10 was used, and the extraction was carried out for one hour. The samples were filtered on Whatman filters (pore size 2.5 µm), then the carbon in the fumigated and non-fumigated extracts were determined with an elemental analyzer for liquid samples (TOC-TN Hypertoc Shimadzu Corp., Kyoto, Japan). The non-fumigated extracts were used to determine as a measure the dissolved organic carbon (SC) and the total dissolved nitrogen (SN). The C and N pools were expressed as mg kg^{-1} dry soil.

The soil respiration was measured for 35 days from the beginning of the incubation. For this analysis, moist soil samples, equivalent to 10 g of dry soil, were weighed in aluminum vessels and an amount of compost, corresponding to the different treatments, was added to the soil. The samples were then placed within airtight glass jars together with a glass vial containing 20 mL of 0.25 M NaOH [27]. Twice a week the vials were changed to new ones. The CO_2 released from soil and trapped by the NaOH was quantified by an elemental analyzer for liquid samples (TOC-VCPH/CPN, Shimadzu, Kyoto, Japan) and expressed as μg C-CO_2 g^{-1} dry soil.

2.3. Soil Microbial Community Structure and Abundance

DNA extraction: A total of 250 mg of each soil sample was extracted for total genomic DNA using the Dnasy® Powerlyzer® PowerSoil® Kit (Qiagen, West Sussex, UK), according to the manufactures' instructions [28]. The purity and concentration of the DNA extraction were quantified by testing the ratio of absorbance at 260 and 280 nm (Infinite® 200 PRO Nano Quant, Tecan, Mannedorf, Switzerland).

PCR-DGGE analysis: To study bacteria (16 s) [29], ammonia-oxidizing bacteria (amoA) [30], and fungi (ITS) [31,32], primers with GC clamps [33] were used to amplify the total soil DNA. PCRs were performed with the TopTaq™ Master Mix Kit (Qiagen, West Sussex, UK).

DGGE analyses were carried out using the D Code Universal Mutation System (BlO RAD, Richmond, CA, USA); 300 ng of PCR product for each sample was loaded onto 6% (*w*/*v*) polyacrylamide gel prepared with solutions containing a denaturant gradient of 40–60% (100% denaturant is 7 M urea and 40% deionized formamide) for the total bacteria and ammonia-oxidizing bacteria. For fungi, 8% (*w*/*v*) polyacrylamide gel with a denaturant gradient of 20–50% was used. Electrophoresis was run at a constant voltage of 75 V for 14 h at 60 °C. Gels were, then, stained in a solution of 1× SYBR Green (Invitrogen Molecular Probes, Eugene, OR, USA) in 1× TAE for 20 min and their images captured in UV transillumination with Gel Doc™ 226 XR apparatus (Bio-Rad).

Profiles were normalized by including a ladder with PCR products obtained from known pure cultures. A cluster analysis was carried out by neighbor-joining algorithms based on the distance matrix (Gel Compare software, version 6.6; Applied Maths, Sint-Martens-Later Belgium). Microbial diversity was analyzed with Gel Compare 6.6 for the following parameters: species richness and the Shannon–Wiener index (H).

Real-time PCR assays: A StepOne Plus Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) was employed to quantify the gene abundance of 16 s *amoA* and ITS. The same pair of primers without GC clamp were performed for *amoA* and ITS as we described in PCR-DGGE. And eub338F and eub518R were used for total bacteria [34]. The assays were performed with a 20 μL PCR amplification mixture containing 10 μL of Fast SYBR® Green Master Mix 2× (Applied Biosystems, Foster City, CA, USA), 50–75 mM of each primer, 10 ng DNA, and nuclease-free water in a MicroAmp Fast Optical 48-Well Reaction Plate. Reactions were performed in triplicate. Initial denaturation was 95 °C for 15 s; then 40 cycles 95 °C for 3 s and 60 °C for 30 s. Data was collected at each annealing step. Copy numbers are reported as gene copies/g·dry soil.

2.4. Statistical Analysis

Differences between the treatments were evaluated by analysis of variance (ANOVA) and the Duncan method ($p < 0.05$) for post hoc multiple comparisons. The Pearson correlation coefficient was employed to detect the correlation between the target parameters. All the gene copy numbers were log-transformed before data analysis to meet normality and homogeneity assumptions.

A multiple linear regression (stepwise method, criteria: probability-of-F-to-enter ≤ 0.050, probability-of-F-to-remove ≥ 0.100) was conducted to assess which biotic and abiotic factors contributed more to the total variance change in soil respiration through the different treatments which are listed in Table 2. The ANOVA, correlation analysis, and multiple linear regression were performed with SPSS 19.0 (IBM, Amonk, NY, USA).

Table 2. Abbreviation of the biotic and abiotic factors assessed in the present study.

	Variables	Units	Abbreviations
Edaphic properties	Clay content	%	CLAY
	Total organic carbon	mg/g	TOC
	Soluble carbon	μg/g	SC
	Microbial biomass carbon	μg/g	MBC
	Soluble nitrogen	μg/g	SN
	Soluble C:N ratio	/	CN
Microbial communities	Abundance of bacteria (qPCR)	gene copies lg/g	B
	Shannon–Wiener index of bacteria	/	BH
	Bacteria richness	numbers of bands	BR
	Abundance of ammonia-oxidizing bacteria (qPCR)	gene copies lg/g	AOB
	Shannon–Wiener index of ammonia-oxidizing bacteria	/	AOBH
	Ammonia-oxidizing bacteria richness	numbers of bands	AOBR
	Abundance of fungi (qPCR)	gene copies lg/g	F
	Shannon–Wiener index of fungi	/	FH
	Fungi richness	numbers of bands	FR
	Fungi/bacteria ratio	/	FB

Structural equation modeling (SEM) allowed us to use a confirmatory approach to test the maximum likelihood of data that fitted the hypothesized path model including soil properties, nutrition sources, and microbial traits as predictors. The predictors were selected from Table 1 if they fulfilled at least one condition described as follows: (a) factors which significantly correlated with soil respiration considered as the main predictor; (b) other variables which significantly correlated with those main predictors. The most parsimonious model was identified by non-significant χ^2 tests ($p \geq 0.05$), a low Akaike Information Criterion (AIC), low Root Mean Square Error of Approximation index (RMSEA ≤ 0.1), low Standardized Root Mean Square Residual index (SRMR ≤ 0.1), and high Comparative Fit Index (CFI ≥ 0.90) [35]. The fit of the path model and structural relationships with data were verified using a SEM analysis conducted with AMOS 22.0 (IBM, SPSS).

3. Results

3.1. Soil Respiration Rate Related with Edaphic Properties

During the incubation period, carbon dioxide release from the Navarra soil was 240.6 $\mu g \cdot g^{-1} \cdot$soil, nearly double that of the Saponi soil, which released 126.2 $\mu g \cdot g^{-1} \cdot$soil. All three treatments exhibited a similar trend in carbon dioxide evolution, with significantly higher emissions in the first two weeks compared to the subsequent two weeks (Figure S1, $p < 0.05$). For the Navarra soil, all three treatments showed a significantly higher carbon dioxide release compared to the control, although no significant differences were observed among the treatments during the first two weeks. After two weeks, the difference in carbon dioxide release between the treatments and the control was no longer evident. In the sandy Saponi soil, the carbon dioxide release was significantly higher in the DAP treatment compared to both compost treatments after two weeks. However, with a longer incubation, the two compost-amended soils showed a significantly higher carbon dioxide release compared to both the DAP treatment and the control soil (Figure S1, $p < 0.05$).

Considering that the addition of nutrients from compost would also bring organic carbon into the treatment, we calculated CO_2 emissions/TOC (Figure S2). CO_2 emissions accounted for 2.6–4.2% in the Navarra soil and 1.2–3.1% in the Saponi soil, respectively, of the TOC through different treatments. Even though the nitrogen addition was of the same level, DAP increased the CO_2 emissions 1.61 (Navarra) and 2.59 (Saponi) times compared to the control soil; the two composts increased 1.4 times (Navarra) and 1.8–2 (Saponi) times. No significant differences were observed between the two compost-based treatments, whereas they differed both from the control and from the DAP-treated soil. Overall, the

CO_2 emissions of all the treatments in the Saponi soil were lower than in the Navarra soil (Figure S2).

Compost addition increased the soluble carbon (SC) in both soils at T0 and T28. At T7, it was higher in ACM and ACFA than DAP in Saponi soil but showed no significant difference in Navarra soil (Figure S3A,B). There was no significant difference between the two soils. At T14, soluble carbon was higher in Navarra soil than Saponi soil (Figure S3A,B). Among the treatments, the soluble carbon of ACM was significantly higher than the control and DAP in sandy soil. The content of soluble nitrogen (SN) trended quite similarly in both soils. It peaked in the DAP treatment, but it was higher in Saponi soil than Navarra soil (Figure S3C,D).

Except for T7, the microbial biomass carbon (MBC) in Navarra soil was higher than Saponi (Figure S3E,F). In Navarra soil, the MBC decreased in the first two weeks in all treatments, and slightly increased in T28 in DAP and ACM (Figure S3E,F). In Saponi soil, all the treatments trended similarly; the MBC peaked at T7 and then slightly decreased with time.

Through all the treatments, the soil respiration rate was related to soil clay content (Table 2). And soil clay content correlated with soluble carbon (SN), microbial biomass carbon (MBC), soluble nitrogen (SN), and C/N ratio (Table 3).

Table 3. Soil respiration rate correlated with clay content (CLAY), soluble carbon (SC), microbial biomass carbon (MBC), soluble nitrogen (SN), and C/N ratio.

	CLAY	**SC**	**MBC**	**SN**	**C/N**
Soil respiration rate	0.423 *	0.112	0.061	0.343	−0.155
p Sig. (2-tailed)	0.016	0.541	0.738	0.055	0.396
CLAY	1.000	0.390 *	0.552 **	−0.165	0.468 **
p Sig. (2-tailed)	/	0.027	0.001	0.367	0.007

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

3.2. Microbial Traits Shifted by Different Treatments

3.2.1. Bacterial Communities

1. Community structure

The dendrogram (Figure S4A) revealed two distinct clusters in the two soils, indicating that the distribution of bacteria differs between them. Similarity in the Navarra soil profile ranged from 70.7% to 92.1%, while in the Saponi soil, it ranged from 82.7% to 96.3% (Figure S4A). The incubation did not significantly affect the composition and structure of bacterial communities in the Navarra soil, as the DGGE profile showed only slight changes over time. In contrast, different clusters were observed in the Saponi soil at various incubation times, indicating that the total microbial community evolved over time. After 28 days, the two compost-amended samples clustered together, setting them apart from the other treatments. Overall, the DAP treatment is often clustered with the control soil, while ACM and ACFA showed greater similarity to each other. Thus, we found that ACM and ACFA had a more significant impact on the bacterial community than the DAP treatment in Saponi soil. Additionally, compared to the Navarra soil, the community structure in the Saponi soil exhibited less stability.

Regarding total bacteria, the ammonia-oxidizing bacteria (AOB) communities differed between the two soils. The similarity of the DGGE profile in the Navarra soil ranged from 78.4% to 97.3%, while in the Saponi soil it ranged from 43.5% to 56.5% (Figure S4B). Interestingly, AOB communities were more closely associated with soil properties than total bacteria. The dominant AOB species varied significantly between the two soils; however, the DGGE profiles remained quite similar across different sampling times and treatments. Notably, the AOB communities in both soils showed considerable similarity across all fertilizer additions, even when compared to the control soil. This suggests that the AOB community may originate from the soils themselves rather than from the fertilizers.

2. Absolute abundance of bacteria similar to ACFA compared

The real-time PCR showed that the absolute abundance of total bacteria from the Navarra soil was significantly higher than that from Saponi (Figure S4C,D). During the incubation period, the DAP and control soil reached the highest cell amount after the first week, and it decreased gradually in both soils (Figure S4C,D). ACM and ACFA trended similarly, increasing smoothly, except for T7 which was lower than the DAP treatment, and reaching the highest value after 28 days. The abundance of ammonia-oxidizing bacteria, checked with the amplification of the *amoA* gene, peaked at T7 in all treatments; the DAP treatment in the Saponi soil almost raised this microbial group up 10 times compared to the other treatments (Figure S4E,F) until the last sampling times, where the concentrations in all samples were almost equal. During all incubation periods, DAP was always significantly higher than the other treatments in both soils from T7 ($p < 0.05$).

3. Correlation between bacteria community and soil properties

Soluble nitrogen significantly correlated with the abundance of ammonia-oxidizing bacteria (Table 4). Soil properties like clay content positively correlated with the abundance, Shannon–Wiener index, and richness of total bacteria communities. Additionally, soluble nitrogen was positively correlated with the abundance of ammonia-oxidizing bacteria, while clay content was negatively correlated with the Shannon–Wiener index of these bacteria (Table 4).

Table 4. Pearson correlation (2-tailed) between the abundance, Shannon–Wiener index (H), and richness (R) of total bacteria (B), ammonia-oxidizing bacteria (AOB), the abundance of fungi (F), clay content, and soluble carbon and nitrogen (SC, SN).

	CLAY	SN	F	SC
B	0.410 *	−0.147	0.467 **	0.279
BH	0.395 *	−0.220	0.460 **	0.074
BR	0.435 *	−0.165	0.434 *	0.105
AOB	−0.065	0.451 **	−0.136	−0.054
AOBH	−0.900 **	0.003	−0.376 *	−0.229
AOBR	−0.427 *	0.019	−0.432 *	0.200
F	0.499 **	−0.365 *	1.000	0.442 *
FH	0.049	−0.261	0.189	0.409 *
FR	0.396 *	−0.224	−0.052	0.501 **

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

3.2.2. Fungal Communities

1. Community structure

The dendrogram of the ITS DGGE profile showed that the fungal profile differed in the two types of soil, as already observed for bacteria. The similarity in the Navarra soil profile ranged from 68.5 to 82.4%, and it was 87.2–96.2 in the Saponi soil (Figure S5A). At T0, after the fertilizer addition, the fungi community structure changed differently in the two soils. In the Navarra soil, the similarity ranged from 74.5 to 76.6. In the Saponi soil, the similarity ranged from 89.1 to 96.2. This means the fertilizer addition influenced the fungal community structure more intensively in Saponi soil than Navarra soil. At T28, in both soils, DAP clustered with CONC, and ACM clustered with ACFA. It appears that ACM and ACFA introduced new fungal species into the system and changed the fungal community structure. Throughout the incubation, fungal communities were more significantly affected by the treatments compared to bacterial communities, especially during the later sampling periods.

2. Abundance of fungi

Like bacteria, the abundance of fungi from the Navarra soil was significantly higher than from the Saponi (Figure S5B,C). Unlike bacteria abundance, fungi trended similarly in

both soils and reached a peak at T14. The ACM and ACFA treatments showed a higher fungal concentration with respect to the DAP and control soils, thus being able to maintain fungal populations in the soil.

3. Correlation between fungi community and soil properties

Soluble nitrogen significantly correlated with the abundance of fungi (Table 3). Clay content and soluble carbon and nitrogen correlated significantly with the communities and compositions of fungi (Table 4). We also found a strong correlation between fungi and bacteria communities (Table 4).

3.2.3. Biotic and Abiotic Factors Contribute to Soil Carbon Dioxide Efflux

Multiple linear regression was employed (stepwise, criteria: probability-of-F-to-enter ≤ 0.050, probability-of-F-to-remove ≥ 0.100) to figure out which biotic and abiotic factors (listed in Table 2) contributed more to the total variance in the change in the soil respiration rate across different treatments. The selected predictors included the abundance and α-diversity of total bacteria and the abundance of ammonia-oxidizing bacteria and fungi that represent microbiota activities. Edaphic properties, which significantly correlated with soil microbial community and respiration rate, were also included (Table 4).

After stepwise selection, model 4 includes the abundance (B) and α-diversity (BH) of the bacteria, soluble nitrogen (SN), and clay content (CLAY) of different treatments (Table 5). In this case, model 4 could explain more than 60% of the soil respiration rate change. Four predictors (the abundance and α-diversity of bacteria, soluble nitrogen, and clay content) could explain 22.22%, 8.26%, 13.58%, and 19.63% each (Table 6).

Table 5. Model summary of multiple linear regression of clay content, soluble nitrogen (SN), abundance (B), and Shannon–Wiener index (BH) of total bacteria.

Model	Predictors	R	R^2
1	B	0.482	0.232
2	B, SN	0.638	0.407
3	B, SN, CLAY	0.704	0.496
4	B, SN, CLAY, BH	0.798	0.637

Table 6. The variance in the main predictors is explained to the total variance changes of soil respiration.

Predictors	Standardized Coefficients	*t*	Sig.	Explained Variance
B	0.461	3588	0.001	22.22%
SN	0.396	3307	0.003	13.58%
CLAY	0.464	3443	0.002	19.63%
BH	−0.417	3232	0.003	8.26%

Structural equation modeling (SEM) was applied to determine direct and indirect effects of microbial communities, different fertilizer sources, and different soil properties on the soil CO_2 released by organizing the dataset into a path relation model. The SEM model confirmed the results obtained from the multiple linear regression, showing that the main predictors remained consistent. The interrelationships among these predictors are illustrated in Figure 2. In addition to their direct effects on CO_2 emissions, clay content and soluble nitrogen significantly influence fungal abundance, which indirectly affects the soil respiration rate. However, the impact of the fungal community is mediated through the bacterial community.

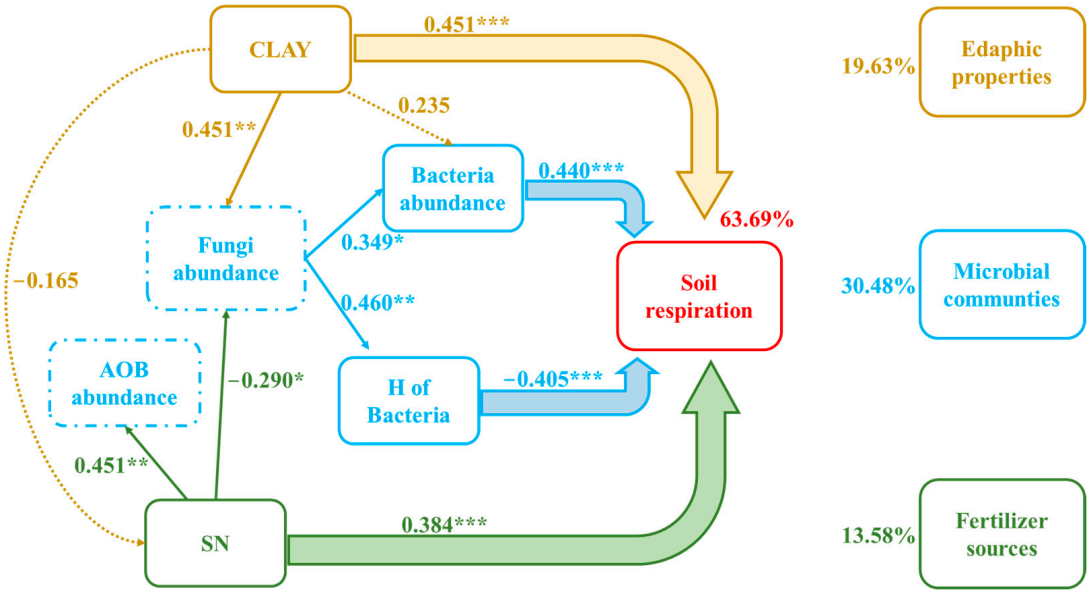

Figure 2. Structural equation model of clay content, soluble nitrogen (SN), and microbial community contributing to soil respiration. Numbers adjacent to arrows are path correlation coefficients; the significant levels are $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.0001$ (***). Width arrows have direct effects, the solid thin arrows are a significant indirect effect, and the dotted thin arrows are not significant. ($\chi^2 = 5254$, df = 9, $p = 0.874$; Standardized Root Mean Square Residual index: RMR = 0.062; Comparative Fit Index: CFI = 1; Root Mean Square Error of Approximation index: RMSEA = 0; Akaike Information Criterion: AIC = 41,254); The different colors distinguish edaphic properties, microbial communities and fertilizer sources. The dash frame indicates an indirect effect on respiration, while the solid frame represents a direct effect.

4. Discussion

This study aimed to investigate the relative roles of various factors contributing to soil respiration following the addition of different fertilizer sources. There are two primary sources of soil CO_2 efflux: rhizospheric respiration and heterotrophic respiration [36]. In this research, we focused solely on the latter. Fertilizer addition led to increased CO_2 efflux, driven by a higher microbial utilization rate of labile carbon, which aligns with previous studies [23,37]. Although compost addition provided more soluble carbon in both soils (Figure S3), inorganic nitrogen input from DAP resulted in higher respiration rates compared to organic nitrogen from ACM and ACFA in both soil types. Notably, significant differences were observed in the sandy soil (Saponi). Total organic carbon levels were similar in the Navarra (0.93%) and Saponi (1.06%) soils, indicating that the increase in respiration rate was triggered by nitrogen addition. Additionally, both compost treatments resulted in less soil organic carbon loss.

The composition of the microbial community can significantly influence microbial respiration, as different microbial functional groups exhibit varying carbon use efficiencies and preferences for carbon type [38,39]. Based on the results from the DGGE fingerprinting and real-time PCR, it appears that while bacterial composition changed only slightly, bacterial abundance peaked at T7. This suggests that the structure and abundance of the bacterial community are key contributors to soil respiration under different treatments. Two explanations may account for these findings. First, bacteria tend to have faster growth rates compared to fungi, making them more responsive to changes in the soil environment. Second, soil bacteria generally have a lower carbon use efficiency than fungi, resulting in higher CO_2 emissions from bacterium-dominated communities compared to those dominated by fungi [40,41]. This study found a positive correlation between the soil respiration rate and bacterial abundance, indicating that increasing bacterial populations may significantly contribute to soil CO_2 emissions, consistent with previous research [39,42].

Through the analysis of various biotic and abiotic factors, multiple linear regression revealed that clay content, soluble nitrogen, and the abundance and α-diversity of bacteria were the primary contributors to soil respiration, more so than other factors. This aligns with studies suggesting that the soil microbial community plays a crucial role in predicting soil respiration rates following multiple fertilization treatments in a single soil type [13]. Our findings are consistent with previous research demonstrating positive correlations between clay content and soil organic matter, as well as between clay content and soil respiration [14,43,44]. However, our results indicate that, in addition to microbial activity, soil texture in agricultural systems can also explain a portion of the soil respiration rate, with its contribution sometimes exceeding that of fertilizer addition.

Liu et al. utilized structural equation modeling (SEM) to gain a comprehensive understanding of how microbial community composition influences soil respiration, taking into account human management, soil properties, and other key microbial drivers identified through their best-fitting distance-based models [13]. Our SEM provided further evidence that not only does the soil microbial community directly affect soil respiration, but edaphic properties, such as clay content, also play a direct role. The Navarra and Saponi soils exhibit typical clay and sandy textures, respectively, with fine fractions (clay + silt) at 87% and 24%. This creates a distinctly different environment for microorganisms. Clay content is crucial in providing diverse soil environments, influencing factors like nutrient availability, water-holding capacity, and cation exchange capacity, all of which ultimately affect soil respiration. A meta-analysis on the response of mineral soil carbon storage noted that clay soils offer more favorable microsites for soil microorganisms to assimilate organic matter [45]. Soil texture also impacts the stability and microbial accessibility of soil organic carbon, a key predictor of mineral soil carbon dynamics [18,38]. Several studies have reported a positive correlation between clay content and carbon stocks across various climatic and edaphic gradients [14,46,47]. Our results indicate that compost addition can maintain a higher level of soluble carbon compared to DAP and control treatments in both soils.

Considering all these factors, we can explain the positive correlation between clay content and the abundance of bacteria and fungi. The SEM model indicated that, over time, clay content had a significant correlation with fungal abundance, which in turn correlated significantly with the abundance and α-diversity of bacteria. This suggests that the influence of clay content on the bacterial community may be mediated by fungi, a relationship that warrants further investigation in future studies. In the current research, while the fungal community did not demonstrate a direct effect on soil respiration, it played a crucial role in interacting with other major predictors. This aligns with the growing body of literature emphasizing the importance of fungal populations in soil functionality, highlighting their role in enhancing microbial interactions and overall soil health [48–51].

Although there are important discoveries revealed by this study, there are also some limitations. First, we selected two soils that have similar nutrient compositions but completely different textures for in-depth study. However, it would be beneficial to conduct research on several types of soils that are prevalent globally to achieve broader application goals. In future research, we can collect soil data and metagenomic data from diverse global habitats and use a meta-analysis to achieve this goal. Second, when adding different fertilizers, the application rates are determined based on consistent nitrogen content, which may also introduce other elements. For example, both ACM and ACFA contain phosphorus, while DAP contains sulfate. These elements may also influence microbial community activity; however, we did not consider these factors in the model. This could be why the existing model only explains 63.69% of the total variance in respiration. Future experiments could clarify the effects of adding these elements on soil respiration by incorporating different gradients.

5. Conclusions

The present study reveals that clay soil (Navarra) exhibits a higher respiration rate compared to sandy soil (Saponi). Fertilizer addition resulted in more pronounced changes in the sandy soil. We found a positive correlation between soil respiration rate and bacterial abundance, confirming that biotic factors play a more significant role in driving soil respiration than abiotic factors. Bacterial communities contributed directly to soil CO_2 efflux, while fungi had an indirect influence. Considering soil respiration as an indicator of soil activity, our comparative studies across different soil types and fertilizer applications suggest that soil texture may have a more substantial impact on respiration than the fertilizer addition itself. In the context of sustainable agricultural production, it is essential to not only address crop nutrient needs but also to determine appropriate fertilizer application rates, timings, and types based on the specific characteristics of different soil types.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112468/s1, Figure S1: Carbon dioxide release from Navarra and Saponi soil through different treatments during the incubation period; Figure S2: Carbon dioxide loss accounted for total organic carbon in Navarra and Saponi soil through different treatments; Figure S3: The mean values of soluble carbon, soluble nitrogen, and microbial biomass in different treatments and different sampling times; Figure S4: DGGE profile and abundance of total bacteria community and ammonia-oxidizing bacteria in different treatments and sampling times; Figure S5: DGGE profile and abundance of fungi community in different treatments and sampling times. Table S1: The correlation matrix was used for the SEM analysis.

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