Catena 214 (2022) 106234

Contents lists available at ScienceDirect

Catena

journal homepage: www.elsevier.com/locate/catena

N cycle in burnt and unburnt soils under different vegetation covers in the Mediterranean region

G. Santini [a], A.G. Ruggiero [a], M.T. Ceccherini [b], L. Santorufo [a,c,*], V. Memoli [a], G. Pietramellara [b], A. De Marco [c,d], A. Giarra [e], G. Di Natale [e,f], M. Trifuoggi [e], R. Barile [g], G. Maisto [a,c]

[a] Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy
[b] DAGRI – Department of Agriculture, Food, Environment and Forestry, University of Florence, P.le delle Cascine, 28 - 50144 Firenze, Italy
[c] BAT Center – Center for Studies on Bioinspired Agro-Environmental Technology, Italy
[d] Department of Pharmacy, University of Naples Federico II, Via Montesano 49, 80131 Naples, Italy
[e] Department Chemistry Science, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy
[f] CeSMA-Centre of Meteorologic and Advanced Technology Services, University of Naples Federico II, Nicolangelo Protopisani Course, 80146 San Giovanni a Teduccio, Naples, Italy
[g] Vesuvius National Park, Via Palazzo del Principe c/o Castello Mediceo, 80044 Ottaviano, NA, Italy

ARTICLE INFO

Keywords:
DNA yield
Eubacterial DNA
N_2-fixers
Nitrifying bacteria
Denitrifying bacteria
Fire

ABSTRACT

The Mediterranean area is a fragile semi-arid ecosystem, characterized by a high level of biodiversity. Due to its climatic conditions, fires are frequent. Fires strongly impact the microbial community of the soil and the biogeochemical cycles of the ecosystem. Although the N cycle is crucial, limited data is available about the effects of fires on the microbial community involved in this cycle in these types of environments. The aim of this research was to evaluate the effects that fire has on the amount of microbial populations involved in the different steps of the N cycle in soils under different vegetation cover. To achieve this, surface soils were collected from unburnt and burnt soil of four plant species (holm oak, pine, black locust, and herbs) from inside the Vesuvius National Park that are typical of the Mediterranean maquis. The soils were analyzed for their main abiotic proprieties (pH, water content, concentrations of organic C, total C, total N, NH_4^+, NO^{2-}, and NO^{3-}). They were also analyzed for their amount of total DNA (DNA yield), eubacterial DNA (16S rDNA), N_2-fixer, ammonia oxidizer, archaea ammonia oxidizer, and denitrifier DNA. The largest amount of microbial populations involved in the N cycle of the unburnt soil was observed in the holm oak soil. Fires slightly affected the soil abiotic properties and the DNA yield of the unburnt soil, but significantly increased the amount of eubacteria, nitrifiers, and denitrifiers. The conditions of the pine and holm oak soil encouraged a faster recovery of the amount of microbial populations involved in the N cycle.

1. Introduction

The Mediterranean maquis of southern Italy is distinguished by different plant species, from low and high maquis to *Quercus ilex* and Pinus spp. forests. Vegetation cover influences the properties of the soil through the distribution of litter. This consequently controls the soil-dwelling microbial populations and, in turn, the soil quality (Rutigliano et al., 2004; Memoli et al., 2021; Santorufo et al., 2021; De Marco et al., 2022).

Additionally, fire frequently and greatly affects the properties of the soil, especially in semi-arid and fragile ecosystems such as the Mediterranean area (Memoli et al., 2020). In fact, fire alters the quantity and biodiversity of microbial populations, due to it warming and changing the physical and chemical properties of the soil (Giuditta et al., 2018; Panico et al., 2020; Memoli et al., 2019). However, different effects on the soil were observed according to how much time had passed since a fire. In particular, Banning and Murphy (2008) found a complete loss of microbial activity immediately after fire. Whereas, Cilliers et al. (2005) found that bacterial and fungal populations were recovered by one year after a fire.

Soil microorganisms, which play a key role in the organic matter degradation of soil, are involved in nutrient cycling (Gruber and Galloway, 2008; Panico et al., 2018). As nitrogen is essential for plants and soil microorganisms (Fitter et al., 2005) and is scarcely available in

* Corresponding author at: Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy.
E-mail address: lucia.santorufo@unina.it (L. Santorufo).

https://doi.org/10.1016/j.catena.2022.106234
Received 14 January 2022; Received in revised form 10 March 2022; Accepted 15 March 2022

soils (Schimel and Bennett, 2004), its cycle is fundamental for the quality of the whole terrestrial ecosystem. The N cycle starts with N-fixers, which reduce N_2 to ammonium (NH_4^+). These are commonly assessed by quantifying the *nif*H genes encoding the nitrogenase (Howard and Rees, 1996). NH_4^+ in soil can be also released by microorganisms involved in the mineralization of N organic compounds. Ammonia oxidizers, such as bacteria (AOB) and archaea (AOA), transform NH_4^+ to NO_2^- (nitrite) and are associated with various ecological niches (Prosser and Nicol, 2012). The amount of these populations is assessed by quantifying the *amo*A genes encoding the A subunit of the NH_3 monooxygenase (Rotthauwe et al., 1997; Leininger et al., 2006). NO_2^- is oxidized to NO_3^- (nitrate) by other populations of nitrifiers. Finally, denitrifiers, reducing NO_3^- to N_2, are assessed by copper-containing nitrite reductase *nir*K genes (Henry et al., 2004), and the N cycle is completed (Espenberg et al., 2018). NO_3^- and NH_4^+ can also be extracted by plants that are able to compete with specific soil microbial populations (Holland et al., 1999).

As microorganisms are sensitive to rapid alterations and perturbations (García-Orenes et al., 2013), the structure and diversity of a soil microbial community provide a lot of information about the overall soil quality and functionality (Zornoza et al., 2009; Santorufo et al., 2021). Therefore, maintaining the different steps of the N cycle primarily depends on the abiotic properties and microbial biodiversity of the soil (Altieri, 1999). Some studies have documented that frequent fires increase the abundance of AOB and decrease that of AOA (Long et al., 2014). Moreover, in Mediterranean area, there was an overall decrease of the amount of microorganisms involved in the N cycle as a consequence of the changes in plant biodiversity that were caused by fire (Pereg et al., 2018).

Therefore, this research aimed to contribute to the current knowledge about the impact of fire on soil microbial populations involved in the N cycle (N_2-fixers, ammonia oxidizers, and denitrifiers), whilst considering the vegetation cover of the investigated sites.

The aims of this research were: (i) to evaluate the possible effects of vegetation cover on the amount of microbial populations involved in the different steps of the N cycle; (ii) to evaluate the possible effects of fire on the populations; and (iii) to highlight the main soil abiotic proprieties that cause possible changes to the microbial community of the soil covered by different plant species that were affected by fire. To achieve this, surface soils were collected from unburnt and burnt areas inside the Vesuvius National Park from four plant species (holm oak, pine, black locust, and herbs) typical of the Mediterranean maquis. The hypotheses behind the aims were: (H1) unburnt soils under holm oak show the highest amount of the investigated microbial populations; (H2) fire causes a decrease in the amount of the investigated microbial populations, especially in soil taken from herbs; and (H3) the contents of organic carbon and water, as well as the concentrations of the different N-compounds, are the main drivers of the variations in the investigated microbial population in soils covered by different plant species that were affected by fire.

2. Materials and methods

2.1. Study area and soil sampling

The research was performed inside the volcanic area of the Vesuvius National Park (Southern Italy), which is covered by flora typical of the Mediterranean regions (Memoli et al., 2018): herbs (mosses, lichens, valerian, *Helichrysum, artemisia* Mill., and many grasses), shrubs (myrtle, laurel, viburnum, brambles, and brooms), and plant species (holm oak and various species of pines). Since 1912, black locust, an invasive species, has been introduced in afforestation plans of the Vesuvius National Park (Memoli et al., 2018) in order to stabilize the Lepti-Vitric andosols (according to the soil classification of the World Reference Base for Soil Resources, 2015). In July 2017, a fire severely burnt level four of the scale proposed by Vega et al. (2013), damaging wide areas of Vesuvius National Park. In fact, the fire affected approximately 50 % of the total extension and 80 % of the total wooded area, completely destroying herbaceous and shrub species and strongly impacting conifers and other trees. The impact of the fire was so severe that entire green areas were devastated and many plants were cut down as they were unstable.

Unburnt and adjacent burnt areas (100 m × 100 m) covered by the same vegetation type, such as holm oak (HO), pine (P), black locust (BL), and herbs and shrubs (H), were chosen to perform the soil sampling during autumn of 2020. Inside each area, three sites were selected (making a total of 24 sites: three sites in the burnt area and three sites in the unburnt area covered by the four vegetation types). At each of them, eight soil cores (depth: 0–10 cm) were sampled. The soil cores of each site were mixed together in order to obtain a homogeneous sample. They were then transported on ice to the laboratory in order to avoid disturbances to the microbial biomass. Following that, the soil samples were sieved (2 mm mesh size) and divided into three groups. One group was kept at room temperature to perform the physico-chemical analyses. The second group was kept at 4 °C to evaluate the contents of NH_4^+, NO_2^-, and NO_3^-. The third group was kept at −80 °C to perform the molecular analyses. All the analyses were performed on triplicates.

2.2. Soil physical–chemical analyses

The soil samples were characterized according to pH, water content (WC), organic C (C_{org}) concentrations, total C and N concentrations, and NH_4^+ and NO_3^- contents. Soil pH was measured, using an electrometric method in soil and a distilled water (1:2.5 = v:v) suspension. The WC was determined gravimetrically by drying fresh soil at 105 °C until a constant weight was reached. C_{org} was measured with a CNS Analyzer (Thermo Finnigan) on soil samples previously treated with HCl (10%) to exclude carbonates. The C and N concentrations were evaluated on oven-dried (105 °C) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples with a CNS Analyzer (Thermo Finnigan). Details for the above-mentioned analyses were reported in Memoli et al. (2018).

NH_4^+ and NO_3^- contents were measured after the extraction of fresh (4 °C) soil samples by UV–Vis spectrophotometry in accordance with the Italian Law DM 13/09/99. Two grams of soil were transferred into a plastic container, and 20 ml of KCl solution (2 M) was added and then stirred for one hour in a rotary shaker at a controlled temperature (22 °C). The solution was left to settle for at least one hour. The supernatant filtered onto filter paper and aliquots of the obtained solution, properly diluted, were built up to 10 ml in a plastic tube for the subsequent measurements. For the NH_4^+ measurement, 0.4 ml of phenol solution (10% w:v in ethanol), 0.4 ml of sodium nitroprusside solution (0.5% w:v in water), and 1 ml of oxidizing solution based on sodium hypochlorite were added. After color development, the NH_4^+ concentration was evaluated using spectrophotometry UV–Vis (Cary50, Varian). This was done by measuring the absorption at a wavelength of 640 nm and by using a calibration curve built in the range 0.025–0.80 mg l^{-1}. To determine the NO_3^- level, 0.2 ml of HCl solution (1 N in water) was added. After shaking, the NO_3^- concentration was evaluated using spectrophotometry UV–Vis (Cary50, Varian). This was done by calculating the differences between the absorption at a wavelength of 220 nm (absorbance NO_3^- and N organic compounds) and 270 nm (absorbance of N organic compounds) and by using a calibration curve built in the range 0.2–5.0 mg l^{-1}. For both the measurements, the QC samples were processed at the beginning, and then every 10th sample in the analytical batch was processed to verify the instrument calibration.

2.3. DNA extraction and quantitative PCR (qPCR) analyses

The functional groups of the soil microbial community were monitored by DNA extraction and subsequent amplification of a specific gene by qPCR (Nannipieri et al., 2003; Panico et al., 2020).

Total soil DNA was extracted using a FastDNA™ SPIN Kit for Soil

Table 1
Quantitative PCR primers used to enumerate bacteria in this study.

Bacterial Groups	Gene	Oligo name	Sequence (5′–>3′)	Reference
Eub	16S rDNA	Eub341f	CCTACGGGAGGCAGCAG	Muyzer et al. (1993), Simmons et al. (2007)
Eub	16S rDNA	Eub515r	TACCGCGGCKGCTGGCA	
Amox	*amo*A	amoA-1F	GGGGGTTTCTACTGGTGGT	Rotthauwe et al. (1997), Mintie et al. (2003), Avrahami et al. (2003)
Amox	*amo*A	amoA-2R	CCCCTCKGSAAAGCCTTCTTC	
Archamox	*amo*A archea	Arch-amoAF	STAATGGTCTGGCTTAGACG	Francis et al. (2005)
Archamox	*amo*A archea	Arch-amoAR	GCGGCCATCCATCTGTATGT	
Den	*nir*K	nirK876f	ATYGGCGGVAYGGCGA	Henry et al. (2004)
Den	*nir*K	nirKr1040	GCCTCGATCAGRTTRTGGTT	
Nfix	*nif*H	PolF	TGCGAYCCSAARGCBGACTC	Poly et al. (2001)
Nfix	*nif*H	PolR	ATSGCCATCATYTCRCCGGA	

(MP Biomedicals) with some modifications according to Ceccherini et al. (2007). DNA yield (ng DNA g^{-1} soil). Purity was evaluated by spectrophotometry (Picodrop™). DNA quality was assessed by agarose gel electrophoresis. Specific primers were used to quantify qPCR eubacterial (16S rDNA), N_2-fixing (*nif*H), ammonia oxidizing (*amo*A), archea ammonia oxidizing (*archamo*A), and denitrifying (*nir*K) sequences in each soil DNA sample. Primer sets and references reporting the cycling conditions are shown in Table 1. In addition, the melting curve conditions were performed from 55 to 95 °C, with increments of 0.5 °C every five seconds. qPCR was performed on 25 μL of the reaction mixture containing 1X iTAQ UNIVERSYBR GREEN SMX 2500 mix (Bio-Rad Laboratories, CA, USA), 10 μM each of the forward and reverse primers, and 40 ng of the template DNA and sterile ddH_2O to reach the appropriate volume. Water was used as a negative control. Each sample was assayed by the CFX96 Touch Real-Time PCR detection system (Bio-Rad laboratories, CA, USA). The run efficiencies ranged from 79.7% to 130.1%, with R2 values ranging from 0.961 to 0.996.

Nanograms of the target sequence were normalized to a gram of soil in order to compare the results among the different functional groups and among soils under different vegetation covers.

2.4. Statistical analyses

The normality of the data distribution was assessed using the Shapiro-Wilk test.

A paired *t*-test was performed to evaluate the significance of the differences in the total DNA yield. It was also used to assess the differences in each functional group of the soil microbial community under the same vegetation cover between burnt and unburnt soils.

The two-way analysis of variance (ANOVA), followed by the *post hoc* test of Holm-Sidak, was performed in order to highlight the direct influences of vegetation cover and fire and their effects on the amount of the DNA yield and the eubacterial (16S rDNA), N_2-fixing (*nif*H), ammonia oxidizing (*amo*A), archea ammonia oxidizing (*archeamo*A), and denitrifying (*nir*K) sequences.

The statistical assays, performed by Systat_SigmaPlot_12.2 software (Jandel Scientific, USA), were considered statistically significant for at least $P < 0.05$. The graphs were created using SigmaPlot 12.2 software (Jandel Scientific, San Rafael, CA, USA).

The ANOVA was performed using the R 4.1.2 programming environment (R Core Team 2016).

3. Results

3.1. Abiotic properties and soil microorganism population in unburnt soils

In unburnt soils, the pH level ranged from 6.81 to 7.49, and water content (WC) ranged from 8.51% to 35.3% w.w.. The concentrations of C_{org}, C, and N, ranged from 1.14% to 4.04% w.w., from 2% to 6.33% w.w., and from 0.15% to 0.29% w.w., respectively (Table 2). The C/N ratios ranged from 8.66 to 21.5 (Table 2). The above soil properties did not statistically vary according to the different vegetation covers, with the exception of the W, which was statistically higher in the holm oak soil. NH_4^+ concentrations ranged from 1.90 to 8.42 mg kg^{-1} w.w., NO_2^- concentrations ranged from 0.34 to 1.29 mg kg^{-1} w.w., and NO_3^- concentrations ranged from 1.85 to 15.65 mg kg^{-1} w.w. (Table 2). The investigated N-compounds did not statistically vary among the different vegetation covers with the exception of NH_4^+, which was statistically lower in the soil taken from herbs (Table 2).

Total DNA yield ranged from 1.68×10^3 to 3.03×10^4 ng g^{-1} w.w., with statistically higher values in holm oak soil (Fig. 1). Bacterial 16S RNA gene sequences (Eub) ranged from 7.91×10^1 to 6.06×10^2 ng g^{-1} w.w., with statistically higher values in the holm oak and pine soils (Fig. 1). *amo*A sequences (Amox) ranged from 4.69×10^{-12} to 5.38×10^{-4} ng g^{-1} w.w., with statistically higher values in the black locust soil (Fig. 1). *Archamo*A sequences (Archamox) ranged from 1.17×10^{-5} to 7.59×10^{-5} ng g^{-1} w.w., with statistically higher values in the holm oak soil (Fig. 1). Finally, *Nir*K sequences (Den) and *nif*H sequences (Nfix) ranged from 2.39×10^{-1} to 9.41×10^{-1} ng g^{-1} w.w. and from 5.82×10^{-4} to 8.59×10^{-3} ng g^{-1} w.w., respectively, with statistically higher values in the holm oak soil (Fig. 1).

In unburnt soils, significant and positive correlations were found between DNA yield, Eub, Archamox, and Den sequences (Fig. 2).

Table 2
Mean values (±s.e.; n = 9 for each plant cover) of pH, water content (WC, expressed as % w.w.), organic carbon content (Corg, expressed as % w.w.), C and N concentrations (expressed as % w.w.), C/N ratio, concentrations of NH_4^+, NO^{2-}, NO^{3-} soils collected under different plant covers (holm oak, HO; pine, P; black locust, BL; herbs, H) inside the Vesuvius National Park (Naples, Southern Italy).

	Unburnt		Burnt					
	HO	P	BL	H	HO	P	BL	H
pH	7.37± 0.02	7.49± 0.22	6.81± 0.11	6.95± 0.04	7.19± 0.06	6.94± 0.04	6.91 ± 0.10	6.94 ± 0.06
WC	35.3 ± 5.53	17.4 ± 4.32	8.50 ± 1.85	11.1 ± 1.30	40.6 ± 2.95	17.6 ± 3.12	19.4 ± 8.84	10.1 ± 1.31
Corg	4.04 ± 0.90	2.06 ± 0.74	2.38 ± 1.45	1.14 ± 0.34	2.24 ± 0.50	3.79 ± 2.20	3.28 ± 1.30	0.53 ± 0.04
C	6.33 ± 1.06	3.09 ± 0.91	3.04 ± 1.37	2.00 ± 0.39	7.07 ± 1.14	3.00 ± 0.23	6.26 ± 2.35	1.04 ± 0.13
N	0.23 ± 0.04	0.18 ± 0.03	0.30 ± 0.055	0.16 ± 0.02	0.41 ± 0.05	0.17 ± 0.02	0.54 ± 0.20	0.08 ± 0.01
C/N	21.5 ± 2.98	14.1 ± 0.99	8.66 ± 3.84	12.1 ± 2.90	12.1 ± 0.49	15.1 ± 2.17	9.01 ± 1.23	12.4 ± 0.76
NH_4^+	8.41 ± 1.35	7.71 ± 1.43	3.37 ± 1.34	1.90 ± 0.27	4.52 ± 1.73	3.22 ± 1.16	2.53 ± 0.98	3.78 ± 1.00
NO^{2-}	0.34 ± 0.02	0.61 ± 0.40	1.28 ± 0.08	0.91 ± 0.06	0.65 ± 0.11	1.06 ± 0.43	0.94 ± 0.25	1.81 ± 0.61
NO^{3-}	1.85 ± 0.08	2.13 ± 0.08	15.6 ± 6.78	15.4 ± 3.65	1.78 ± 0.04	5.73±3.62	7.49±5.44	7.88 ± 5.64

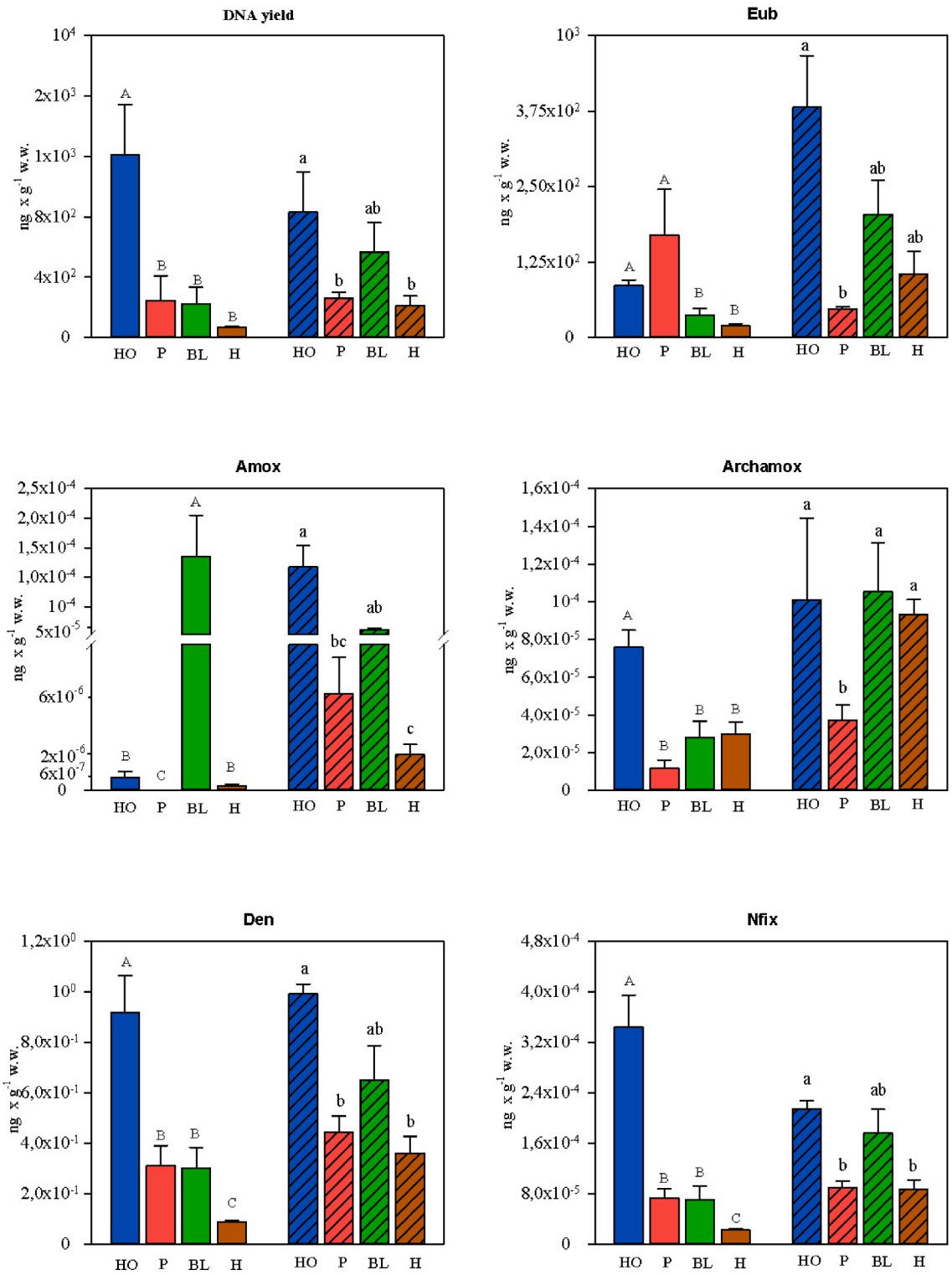

Fig. 1. Mean values (±s.e.; n = 9 for each vegetation cover) of total DNA (DNA yield), Eubacterial DNA (Eub), ammonia oxidizers (Amox), archea ammonia oxidizers (Archamox), denitrifiers (Den) and N_2-fixers (Nfix) in unburnt (no pattern) and burnt (coarse pattern) soils collected under different vegetation covers (holm oak, HO; pine, P; black locust, BL; herbs, H) inside the Vesuvius National Park (Naples, Southern Italy). Different capital and small letters indicate statistically significant differences (ANOVA test; $P < 0.05$) in each population among different vegetation covers, respectively, in unburnt and burnt soils.

Moreover, significant and negative correlations were found between NO_3^- concentrations and DNA yield, Eub, Den, and Nfix sequences (Fig. 3). Significant positive correlations were found between NH_4^+ concentrations and DNA yield and Den sequences. Moreover, positive correlation was observed between N concentration and Den (Fig. 3).

3.2. *Abiotic properties and soil microorganism population in burnt soils*

In burnt soil, pH levels ranged from 6.91 to 7.19, and WC ranged from 10.14% to 40.60% w.w., with values statistically lower in the soil taken from herbs (Table 2). Concentrations of C_{org}, C, and N ranged from 0.53 to 3.8 % w.w., from 1.04% to 7.06% w.w., and from 0.07% to

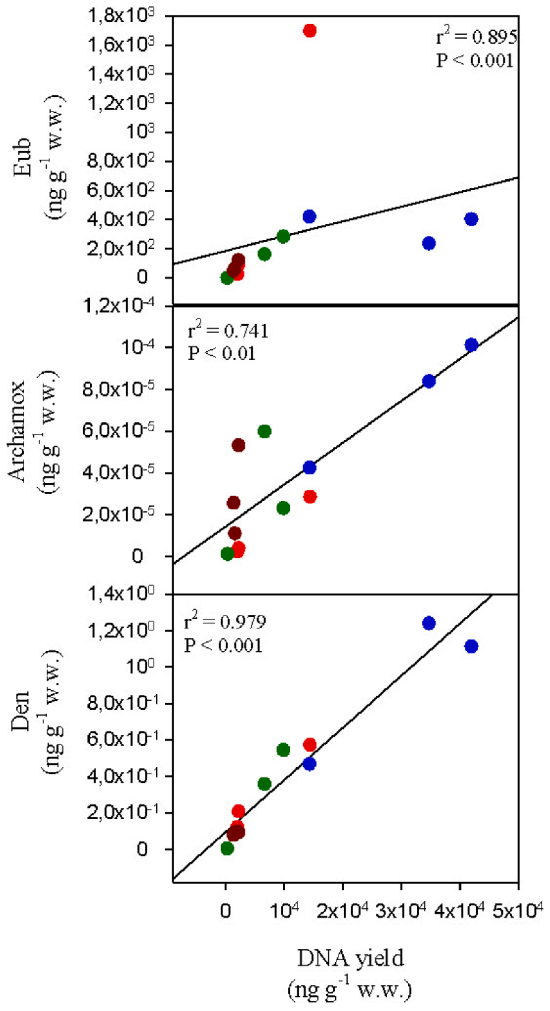

Fig. 2. Regression lines (Spearman's correlations) between amounts of Eubacterial DNA (Eub), archea ammonia oxidizers (Archamox) and denitrifiers (Den) and total DNA (DNA yield) in unburnt soils (blue: holm oak; red: pine; green: black locust; brown: herbs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

0.54% w.w., respectively (Table 2). C/N ratios ranged from 9 to 15.0. C_{org} concentrations and C/N ratios did not statistically vary according to vegetation cover (Table 2). Whereas, C and N concentrations were statistically higher in the black locust soil and lower in the soil taken from herbs (Table 2). NH_4^+ concentrations ranged from 2.53 to 4.52 mg kg^{-1} w.w., NO_2^- concentrations ranged from 0.65 to 1.81 mg kg^{-1} w.w., and NO_3^- concentrations ranged from 1.78 to 7.89 mg kg^{-1} w.w. (Table 2). They did not vary among soils under different covers (Table 2). Total DNA yield ranged between 5.31×10^3 and 2.08×10^4 ng g^{-1} w.w., and did not vary significantly according to vegetation cover (Fig. 1). Eub sequences ranged from 1.87×10^2 to 1.52×10^3 ng g^{-1} w.w., with values statistically higher in the holm oak soil and statistically lower in the pine soil (Fig. 1). Amox sequences ranged from 1.95×10^{-6} to 4.18×10^{-4} ng g^{-1} w.w., and were statistically higher in holm oak soil and statistically lower in the herb soil (Fig. 1). Archamox sequences ranged from 3.70×10^{-5} to 1.06×10^{-4} ng g^{-1} w.w., and were statistically lower in pine soil (Fig. 1). Den and Nfix sequences ranged from 3.35×10^{-1} to 9.93×10^{-1} ng g^{-1} w.w. (Fig. 1) and from 2.18×10^{-3} to 5.26×10^{-3} ng g^{-1} w.w., respectively, and were statistically higher in the holm oak soil and lower in the herb soil (Fig. 1).

In the burnt soils, significant and positive correlations were found between N concentration and DNA yield and Den and Nfix sequences, as well as between NH_4^+ concentrations and Eub sequences (Fig. 4).

3.3. Comparison between soil microorganism populations in burnt and unburnt soils

The comparison of the quantity of the investigated microbial populations (Fig. 1 and Table 4) highlighted that Eub sequences were statistically higher in burnt soils than in unburnt soils of the holm oak and black locust (holm oak: 1.52×10^3 and 3.54×10^2 in burnt and unburnt soils, respectively; black locust: 8.23×10^2 and 1.50×10^2 in burnt and unburnt soils, respectively). Amox sequences were statistically higher in unburnt soils from the black locust (4.73×10^{-5} and 5.38×10^{-4} in burnt and unburnt soils, respectively) and in burnt soils from pines and herbs (pine: 5.27×10^{-6} and 4.68×10^{-12} in burnt and unburnt soils, respectively; herbs: 1.95×10^{-6} and 2.24×10^{-7} in burnt and unburnt soils, respectively). Archamox sequences were statistically higher in burnt soils from pine, black locust, and herbs (pine: 3.70×10^{-5} and 1.70×10^{-5} in burnt and unburnt soils, respectively; black locust: 9.86×10^{-5} and 2.80×10^{-5} in burnt and unburnt soils, respectively; herbs: 9.45×10^{-5} and 2.99×10^{-5} in burnt and unburnt soils, respectively). Finally, Nfix and Den sequences were statistically higher in burnt soils from black locust and herbs (Nfix under black locust: 3.94×10^{-3} and 1.75×10^{-3} in burnt and unburnt soils, respectively; Nfix under herbs: 2.18×10^{-3} and 5.82×10^{-4} in burnt and unburnt soils, respectively; Den under black locust: 6.52×10^{-1} and 3.03×10^{-1} in burnt and unburnt soils, respectively; Den under herbs: 3.35×10^{-1} and 2.39×10^{-1} in burnt and unburnt soils, respectively).

3.4. Effects of vegetation cover and fire on soil microorganism populations

The two-way ANOVA analysis highlighted that the amount of DNA in all the investigated microbial populations was affected by vegetation cover, with values, on average, higher in the holm oak and pine soils. Moreover, Eub, Archamox, and Den sequences were also enhanced by fire. Finally, the DNA quantity, with the exception of those related to Archamox and Den, was affected by the interaction between both vegetation cover and fire (Table 3). In particular, fire occurrence further emphasized the differences in the amount of Eub already existing among various vegetation covers. Whereas, it did not significantly impact the differences in DNA yield, Amox, and Nfix that already existed among those vegetation covers (Table 3).

4. Discussion

In both the unburnt and burnt soils of the investigated area of the Vesuvius National Park, DNA yield and specific microbial sequence quantities varied widely among soils that were under different vegetation covers. The highest DNA yield, representing the total microbial biomass (Fornasier et al., 2014), was found in unburnt soils taken from under the holm oak plant (approximately five-fold higher than in soil from under pines and black locust, and 18-fold higher than that taken from under herbs). This was likely due to the greater litter accumulation compared to the soils from under the other vegetation covers. Thus, the wide heterogeneity of micro niches could have enhanced the microbial biomass and its biodiversity (Prescott and Grayston 2013; Picariello et al., 2021). Moreover, the higher water availability in holm oak soil (approximately two-fold higher than in the pine soil, four-fold higher than in the black locust soil, and three-fold higher than in the herb soil), could be responsible for the enhanced microbial growth. In fact, soil water condition is fundamental in controlling the microbial growth, especially in arid and semi-arid ecosystems (Griffiths and Philippot, 2013; Liu et al., 2010) such as the Vesuvius National Park (Memoli et al., 2018). Compared to the other vegetation, the holm oak soil was in the best condition. This was confirmed by it containing the highest amount of the microbial populations involved in the N cycle (specifically, the amount of Eub was, on average, two-fold higher than in the other types of vegetation; Archamox was, on average, four-fold higher than the other types of vegetation; Nfix was, approximately, five-fold higher than

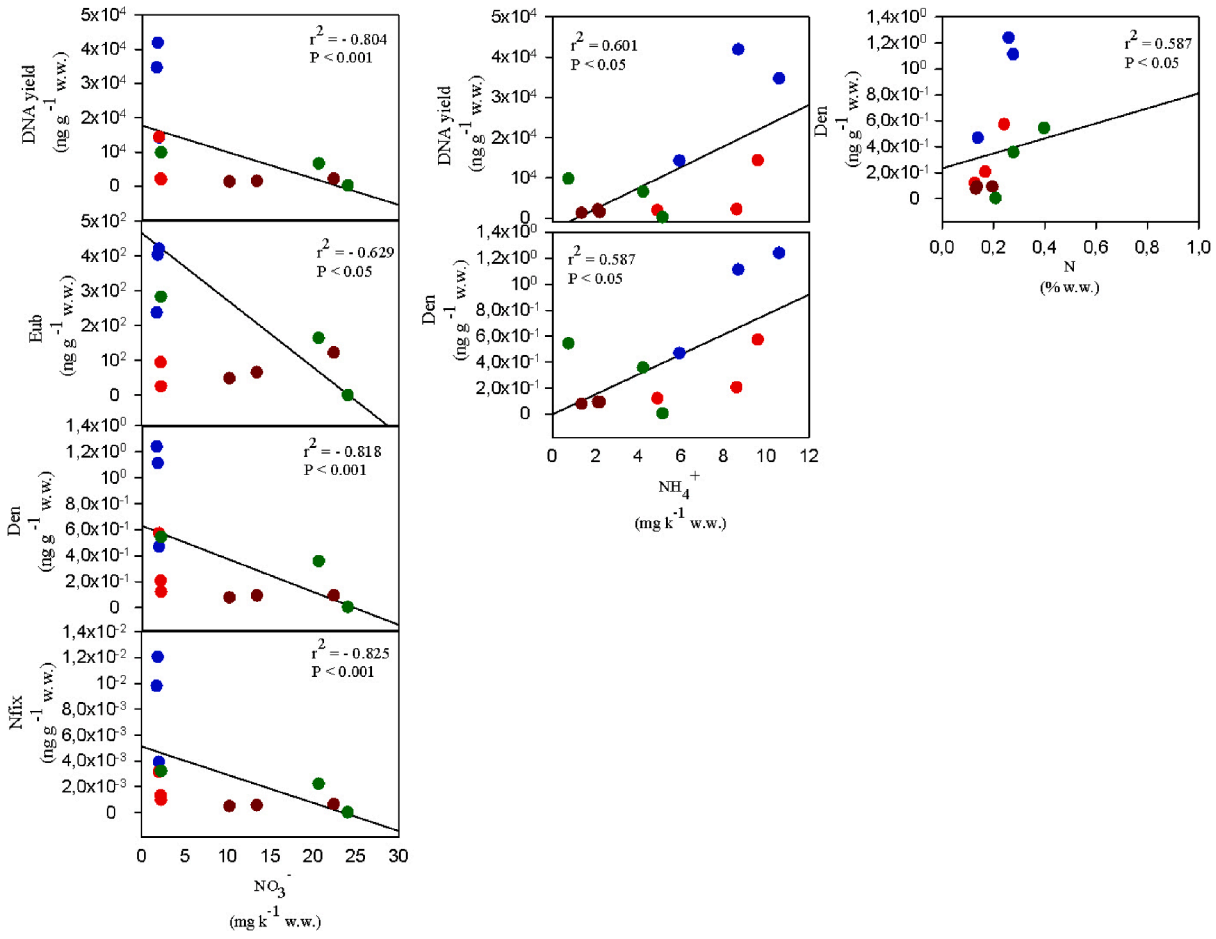

Fig. 3. Regression lines (Spearman's correlations) between amounts of total DNA (DNA yield), Eubacterial DNA (Eub), ammonia oxidizers (Amox), denitrifiers (Den) and N_2-fixers (Nfix) and concentrations of NO^{3-}, NH_4^+ and total N in unburnt soils (blue: holm oak; red: pine; green: black locust; brown: herbs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the pine and black locust soils, and 15-fold higher than herb soil; Den was approximately three-fold higher than pine and black locust soils, and 11-fold higher than the herb soil), with only the exception of Amox (Banning and Murphy, 2008; Goberna et al., 2012; Cobo-Díaz et al., 2015). Notably, the largest amount of Amox in the unburnt soil being taken from the black locust soil (approximately 10^2-fold higher than the holm oak and herb soils, and 10^8-fold higher than the pine soil) could be due to the symbiotic relationship between its roots and N fixers (Panico et al., 2020). This determines a high availability of NH_4^+, which is a resource for Amox (Wendeborn, 2019; Di et al., 2009). The comparison between unburnt and burnt soils highlighted that fire affected the Amox amounts differently, according to the surrounding vegetation. This was because it inhibited the Amox development in black locust soil and stimulated it in holm oak, pine, and herb soils. It is likely that fires inhibited the biological nitrogen fixation in black locust soil, but created favorable conditions in the other investigated plant species (Tierney et al., 2019).

An overall evaluation highlighted that in the unburnt soils, the amount of Eub, Archamox, and Den significantly increased with the DNA yield increase. Moreover, according to the correlations that were found, soil NH_4^+, NO_3^-, and total N concentrations seemed to be effective drivers of the development of specific microbial populations involved in N cycle. In fact, Den seemed to be stimulated by the increase of N concentrations in soils (Castaldi and Aragosa, 2002). Furthermore, being involved in nitrate degradation, the denitrifiers caused the NO_3^- decrease associated with the production of N reduced forms (Valera and Alexander, 1961; Shtratnikova et al., 2015; Zhang et al., 2020; Florio et al., 2021). Instead, the highest soil concentrations of NO_3^-, widely absorbable by soil microorganisms, seemed to inhibit the development of Nfix (Voisin et al., 2002).

Immediately after a fire, the soil abiotic properties slightly changed compared to the pre-fire conditions in the whole investigated area, suggesting that fire affected them to a similar extent regardless of plant species (Memoli et al., 2021). By contrast, three years after a fire, the effects on microbial populations involved in the N cycle were still evident (Borgogni et al., 2019). In particular, the significant differences in DNA yield, Amox, and Nfix sequences existing in unburnt soils covered by different plant species, were hidden by fire. The significant differences in the quantities of Archamox and Den were also rectified by the interaction between the vegetation and fire. Therefore, the soil properties that drove their quantities seemed to be affected by the different vegetations (*i.e.*, NO_3^- availability) rather than by fire. Instead, the differences in the amount of Eub post-fire were likely due to changes in NH_4^+ level that had enhanced them (Pereg et al., 2018). Whereas, those observed before a fire were mainly due to the NO_3^- level. It is well known that fire indirectly influences the survival, recolonization, and distribution of microbial populations (Velasco, et al., 2009; Griffiths and Philippot, 2013) through changes in soil properties (Bissett and Parkinson, 1980; Monleon et al., 1997; Certini, 2005). Moreover, in the burnt soils that were investigated, the amount of DNA yield, Den, and Nfix were also related to the total N concentrations (Pereg et al., 2018).

The comparison of each population in unburnt and burnt soils under the same vegetation cover highlighted that although the DNA yield did not significantly vary, the relative amount of each investigated population involved in the N cycle significantly varied. Moreover, many of them, after three years, had not yet reached the values of the pre-fire

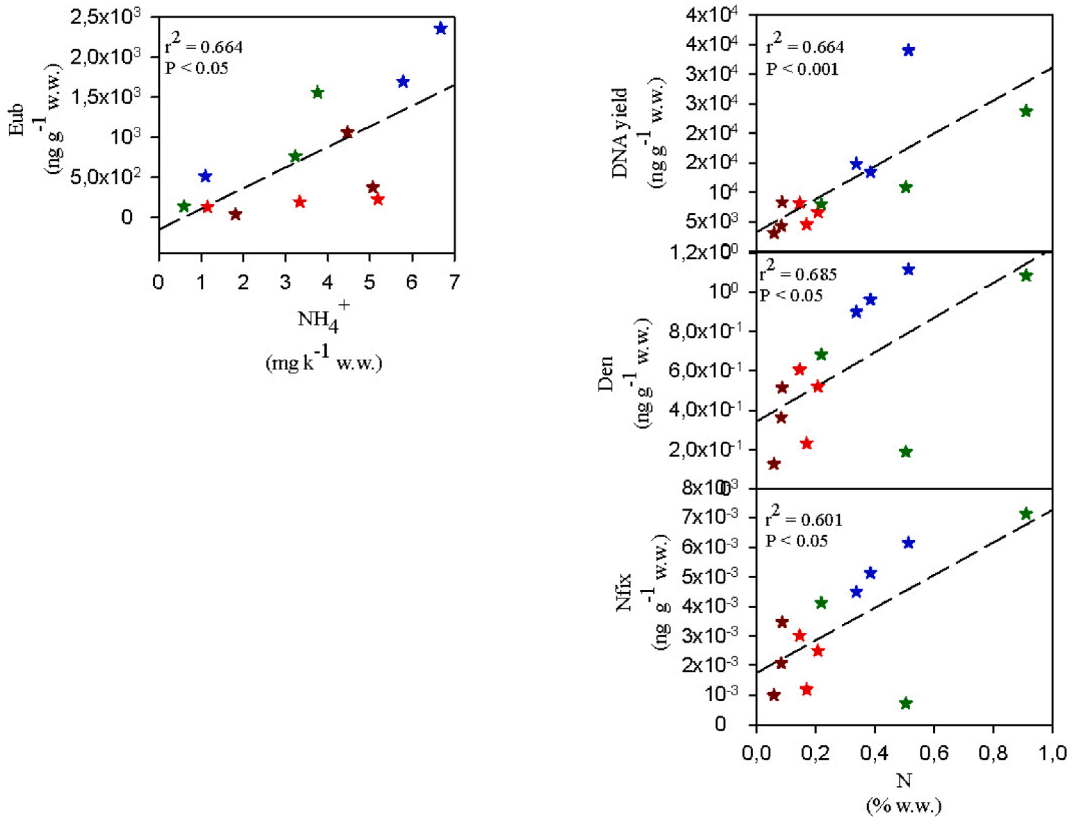

Fig. 4. Regression lines (Spearman's correlations) between amounts of total DNA (DNA yield), Eubacterial DNA (Eub), denitrifiers (Den) and N_2-fixers (Nfix) and concentrations of NH_4^+ and total N in burnt soils (blue: holm oak; red: pine; green: black locust; brown: herbs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Coefficients (F values) of the two-way ANOVA performed on total DNA (DNA yield), Eubacterial DNA (Eub), ammonia oxidizers (Amox), archea ammonia oxidizers (Archamox), denitrifiers (Den) and N_2-fixers (Nfix) in relationship to plant covers (Plant), fire occurrence (Fire) and their interactions (Plant × Fire).

	Plant	Fire	Plant × Fire
DNA yield	31.0***	0.19	5.77**
Eub	4.49**	10.5**	9.39***
Amox	2.92*	0.04	5.71**
Archamox	5.40**	16.7***	1.18
Den	29.4***	11.9***	1.05
Nfix	24.4***	0.650	7.71***

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 4
Coefficients (t-values) of the *t*-test performed on the soil abiotic (pH; water content: WC; organic carbon content: Corg; C and N concentrations; C/N ratios; concentrations of NH_4^+, NO^{2-}, NO^{3-}) and biotic (total DNA: DNA yield; Eubacterial DNA: Eub; ammonia oxidizers: Amox; archea ammonia oxidizers: Archamox; denitrifiers: Den; N_2-fixers: Nfix) properties to evaluate statistically significant differences between unburnt and burnt soils collected under the same plant cover, inside the Vesuvius National Park (*t*-test analysis).

	HO	P	BL	H
pH	2.70	15	0.672	0.248
WC	0.851	0.025	1.21	0.52
Corg	1.75	0.75	0.460	1.75
C	0.471	0.103	1.18	2.37
N	2.73	0.140	1.20	3.51*
C/N	15	0.395	0.087	0.126
NH_4^+	1.18	2.43	0.509	1.81
NO^{2-}	2.79*	0.757	1.31	1.47
NO^{3-}	13	0.993	0.938	1.11
DNA yield	0.90	0.080	1.55	2.27
Eub	3.98**	1.5	3.26**	1.95
Amox	3.46**	2.30*	1.66	3.00**
Archamox	0.687	2.74*	2.98**	6.43***
Den	0.51	1.30	2.21*	4.05**
Nfix	2.32*	0.89	2.42*	4.13***

*P < 0.05; **P < 0.01; ***P < 0.001.

conditions and they could not be considered stabilized. An overall evaluation highlighted that, regardless of vegetation covers, nitrifying microorganisms (*i.e.*, Amox and Archamox) had still increased three years after the fire. These findings are in accordance with Dannenmann et al. (2011), who detected a growth rate of nitrification in post-fire conditions. The findings also correlate with Zhang et al. (2018), who found an increase in the amount of bacteria and archaea related with *amo*A genes after a prescribed fire. However, different behaviors of the population involved in N cycle were detected in soils under different vegetation covers. In fact, herb and black locust soils showed significant differences in all the microbial populations, with the exception of Eub and Amox, respectively. Therefore, it can be theorized that these vegetation covers created soil conditions that stimulated the development of the microbial populations involved in the N cycle, distancing them from the unburnt conditions (Panico et al., 2020). Conversely, major resilience of the investigated populations involved in N cycle was observed in the pine and holm oak soils. In fact, Eub, Den, and Nfix reached their pre-fire quantities in the pine soil. Archamox and Den also returned to their pre-fire quantities in the holm oak soil. The pine soil was the fastest to return to its pre-fire condition. This could be due to the thickening of the pine needles, resulting in an enrichment of nutrients, which are resources for the microbial populations (Knicker, 2007; Pereira et al., 2012).

As the soils of the whole investigated area had still not reached the

pre-fire conditions after three years, management practices allowing for the planting of native species less susceptible and more resistant to fire are necessary. These interventions are of primary importance, especially in the areas not previously covered by trees. This will prevent erosion phenomena, restore the naturalistic value of the National Park, and create the conditions for a rapid settlement of the vegetation.

5. Conclusions

In the investigated unburnt area, different vegetation covers significantly affected the DNA yield and the amount of the microbial populations involved in N cycle. In particular, the highest quantities were observed in holm oak soil because of the wide heterogeneity of soil micro niches created by the large amount of litter accumulation, validating the first hypothesis.

Fires slightly affected the soil abiotic properties and the DNA yield. Whereas, an overall evaluation highlighted that the amounts of Eub, Den, and Archamox increased. This conclusion highlighted that the second hypothesis was only partially validated, as a reduction in the amount of the investigated microbial populations was determined.

The main drivers of microbial population quantities in unburnt soils were the concentrations of NO_3^-, NH_4^+, and total N concentrations. Those in the burnt soils were NH_4^+ and total N concentrations, also the third hypothesis was partially validated. In any case, the fundamental role of the contents of organic carbon and water in controlling the amount of the investigated microbial populations cannot be ignored, at least in unburnt holm oak soil.

An overall conclusion highlighted that a faster recovery of the amount of the microbial populations involved in the N cycle was observed in soils covered by pine and holm oak compared to soil under herbs and black locust, which remained stable three years after the fire. Therefore, further investigations are needed to follow the temporal development of the microflora and to estimate the time required to reach the amount of the microbial populations involved in the N cycle of the pre-fire conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The research was funded by the collaboration of the Biology Department of University Federico II of Naples and the Vesuvius National Park within the “Azione di Sistema - Impatto antropico da pressione turistica nelle aree protette: interferenze su territorio e biodiversità” funded by “Ministero dell’Ambiente e della Tutela del Territorio e del Mare”, Direttiva Conservazione della Biodiversità.

The authors wish to thank Miss Melissa Gurgone for English revision.

References

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. In: Paoletti, M. G. (Ed.), Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes. Elsevier, pp. 19–31.

Avrahami, S., Liesack, W., Conrad, R., 2003. Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. Environ. Microbiol 5, 691–705.

Banning, N.C., Murphy, D.V., 2008. Effect of heat-induced disturbance on microbial biomass and activity in forest soil and the relationship between disturbance effects and microbial community structure. Appl. Soil Ecol. 40 (1), 109–119. https://doi.org/10.1016/j.apsoil.2008.03.011.

Bissett, J., Parkinson, D., 1980. Long-term effects of fire on the composition and activity of the soil microflora of a subalpine, coniferous forest. Can. J. Bot. 58 (15), 1704–1721.

Borgogni, F., Lavecchia, A., Mastrolonardo, G., Certini, G., Ceccherini, M.T., Pietramellara, G., 2019. Immediate- and short-term wildfire impact on soil microbial diversity and activity in a mediterranean forest soil. Soil Sci. 184-2-35-42.

Castaldi, S., Aragosa, D., 2002. Factors influencing nitrification and denitrification variability in a natural and fire-disturbed mediterranean shrubland. Biol. Fert. Soils 36 (6), 418–425. https://doi.org/10.1007/s00374-002-0549-2.

Ceccherini, M.T., Ascher, J., Pietramellara, G., Mocali, S., Viti, C., Nannipieri, P., 2007. The effect of pharmaceutical waste-fungal biomass, treated to degrade DNA, on the composition of eubacterial and ammonia oxidizing populations of soil. Biol. Fert. Soils 44 (2), 299–306.

Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143 (1), 1–10.

Cilliers, C.D., Botha, A., Esler, K.J., Boucher, C., 2005. Effects of alien plant management, fire and soil chemistry on selected soil microbial populations in the Table Mountain National Park. South Africa. S. Afr. J. Bot 71 (2), 211–220.

Cobo Diaz, J.F., Fernández-González, A.J., Villadas P.J., Robles A.B., Toro N., Fernández-López M., 2015. Metagenomic assessment of the potential microbial nitrogen pathways in the rhizosphere of a Mediterranean forest after a wildfire. Microb. Ecol. 69,895–904.

Dannenmann, M., Willibald, G., Sippel, S., Butterbach-Bahl, K., 2011. Nitrogen dynamics at undisturbed and burned Mediterranean shrublands of Salento Peninsula, Southern Italy. Plant Soil 343 (1-2), 5–15. https://doi.org/10.1007/s11104-010-0541-9.

De Marco, A., Panico, S.C., Memoli, V., Santorufo, L., Zarrelli, A., Barile, R., Maisto, G., 2022. Differences in soil carbon and nitrogen pools between afforested pine forests and natural shrublands in a Mediterranean area. Appl. Soil Ecol. 170, 104262. https://doi.org/10.1016/j.apsoil.2021.104262.

Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J. Z., 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat. Geosci. 2 (9), 621–624. https://doi.org/10.1038/ngeo613.

Espenberg, M., Truu, M., Mander, Ü., Kasak, K., Nõlvak, H., Ligi, T., Truu, J., 2018. Differences in microbial community structure and nitrogen cycling in natural and drained tropical peatland soils. Sci. Rep. 8 (1), 4742.

Fitter, A.H., Gilligan, C.A., Hollingworth, K., Kleczkowski, A., Twyman, R.M., Pitchford, J.W., 2005. Biodiversity and ecosystem function in soil. Funct. Ecol. 19 (3), 369–377.

Florio, A., Marechal, M., Legout, A., Creuse des Chatelliers, C., Gervaix, J., Didier, S., Zeller, B., Le Roux, X., 2021. Le Roux, Influence of biological nitrification inhibition by forest tree species on soil denitrifiers and N2O emissions. Soil Biol. Biochem. 155, 108164. https://doi.org/10.1016/j.soilbio.2021.108164.

Fornasier, F., Ascher, J., Ceccherini, M.T., Tomat, E., Pietramellara, G., 2014. A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. Ecol. Ind. 45, 75–82.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102 (41), 14683–14688.

García-Orenes, F., Morugán-Coronado, A., Zornoza, R., Scow, K., Herrera-Estrella, A., 2013. Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agroecosystem. PLoS One 8 (11), e80522. https://doi.org/10.1371/journal.pone.0080522.

Giuditta, E., Coenders-Gerrits, A.M.J., Bogaard, T.A., Wenninger, J., Greco, R., Rutigliano, F.A., 2018. Measuring changes in forest floor evaporation after prescribed burning in Southern Italy pine plantations. Agric. For. Meteorol. 256-257, 516–525.

Goberna, M., García, C., Insam, H., Hernández, M.T., Verdú, M., 2012. Burning fire-prone Mediterranean shrublands: immediate changes Nitrogen cycle and bacterial diversity after a wildfire 903 in soil microbial community structure and ecosystem functions. Microb. Ecol. 64, 242–255.

Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37 (2), 112–129. https://doi.org/10.1111/j.1574-6976.2012.00343.x.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451 (7176), 293–296.

Henry, S., Baudoin, E., López-Gutiérrez, J.C., Martin-Laurent, F., Brauman, A., Philippot, L., 2004. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. J. Microbiol. Methods 59 (3), 327–335.

Holland, E.A., Dentener, F.J., Braswell, B.H., Sulzman, J.M., 1999. Contemporary and pre-industrial global reactive nitrogen budgets. Biogeochemistry 46 (1–3), 7–43.

Howard, J.B., Rees, D.C., 1996. Structural basis of biological nitrogen fixation. Chem. Rev. 96 (7), 2965–2982.

Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85 (1), 91–118. https://doi.org/10.1007/s10533-007-9104-4.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammoniaoxidizing prokaryotes in soils. Nature 442 (7104), 806–809.

Liu, B., Mørkved, P.T., Frostegård, Å., Bakken, L.R., 2010. Denitrification gene pools, transcription and kinetics of NO, N2O and N2 production as affected by soil pH. FEMS Microbiol. Ecol. 72, 407–417.

Long, X., Chengrong, C., Zhihong, X., Ji-zheng, H., 2014. Shifts in the abundance and community structure of soil ammonia oxidizers in a wet sclerophyll forest under long-term prescribed burning. Sci. Total Environ. 470–471, 578–586. https://doi.org/10.1016/j.scitotenv.2013.10.011.

Memoli, V., Eymar, E., García-Delgado, C., Esposito, F., Santorufo, L., De Marco, A., Barile, R., Maisto, G., 2018. Total and fraction content of elements in volcanic soil: natural or anthropogenic derivation. Sci. Total Environ. 625, 16–26.

Memoli, V., De Marco, A., Esposito, F., Panico, S.C., Barile, R., Maisto, G., 2019. Seasonality, altitude and human activities control soil quality in a national park surrounded by an urban area. Geoderma 337, 1–10.
Memoli, V., Panico, S.C., Santorufo, L., Barile, R., Di Natale, G., Di Nunzio, A., Toscanesi, M., Trifuoggi, M., De Marco, A., Maisto, G., 2020. Do wildfires cause changes in soil quality in short term? Int. J. Environ. Res. Public Health 17, 5343. https://doi.org/10.3390/ijerph17155343.
Memoli, V., Santorufo, L., Panico, S.C., Barile, R., Di Natale, G., Trifuoggi, M., De Marco, A., Maisto, G., 2021. Stability of mediterranean burnt soils under different plant covers. CATENA 206, 105581. https://doi.org/10.1016/j.catena.2021.105581.
Mintie, A.T., Heichen, R.S., Cromack,, K., Myrold, D.D., Bottomley, P.J., 2003. Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade mountains. Appl. Environ. Microbiol. 69 (6), 3129–3136.
Monleon, V.J., Cromack, Jr., K., Landsberg, J.D., 1997. Short- and long-term effects of prescribed underburning on nitrogen availability in ponderosa pine stands in central Oregon. Can. J. For. Res. 27 (3), 369–378.
Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reactionamplified genes coding for 16s rRNA. Appl. Environ. Microbiol. 59 (3), 695–700.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.
Panico, S.P., Memoli, V., Esposito, F., Maisto, G., De Marco, A., 2018. Plant cover and management practices as drivers of soil quality. Appl. Soil Ecol. 129, 34–42. https://doi.org/10.1016/j.apsoil.2018.05.001.
Panico, S.P., Ceccherini, M.T., Memoli, V., Maisto, G., Pietramellara, G., Barile, R., De Marco, A., 2020. Effects of different vegetation types on burnt soil properties and microbial communities. Int. J. Wildland Fire 29, 628–636. https://doi.org/10.1071/WF19081.
Pereg, L., Mataix-Solera, J., McMillan, M., García-Orenes, F., 2018. The impact of post-fire salvage logging on microbial nitrogen cyclers in Mediterranean forest soil. Sci. Total Environ. 619-620, 1079–1087.
Pereira, P., Ubeda, X., Martin, D.A., 2012. Fire severity effects on ash chemical composition and water-extractable elements. Geoderma 191, 105–114. https://doi.org/10.1016/j.geoderma.2012.02.005.
Picariello, E., Baldantoni, D., Izzo, F., Langella, A., De Nicola, F., 2021. Soil organic matter stability and microbial community in relation to different plant cover: a focus on forests characterizing Mediterranean area. Appl. Soil Ecol. 162, 103897. https://doi.org/10.1016/j.apsoil.2021.103897.
Poly, F., Ranjard, L., Nazaret, S., Gourbière, F., Monrozier, L.J., 2001. Comparison of nifH gene pools in soils and soil microenvironments with contrasting properties. Appl. Environ. Microbiol. 67 (5), 2255–2262. https://doi.org/10.1128/AEM.67.5.2255-2262.2001.
Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: current knowledge and research needs. For. Ecol. Manag. 309, 19–27.
Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. TIM 20 (11), 523–531. https://doi.org/10.1016/j.tim.2012.08.001.
Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammoniaoxidizing populations. Appl. Environ. Microbiol. 63 (12), 4704–4712.
Rutigliano, F.A., D'Ascoli, R., Virzo De Santo, A., 2004. Soil microbial metabolism and nutrient status in a Mediterranean area as affected by plant cover. Soil Biol. Biochem. 36 (11), 1719–1729.
Santorufo, L., Memoli, V., Panico, S.C., Santini, G., Barile, R., Di Natale, G., Trifuoggi, M., De Marco, A., Maisto, G., 2021. Early post-fire changes in properties of Andosols within a Mediterranean area. Geoderma 394, 115016. https://doi.org/10.1016/j.geoderma.2021.115016.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85(3), 591–602. <https://doi.org/https://doi.org/10.1890/03-8002>.
Shtratnikova, V.Y., Kudryakova, N.V., Kudoyarova, G.R., Korobova, A.V., Akhiyarova, G. R., Danilova, M.N., Kusnetsov, V.V., Kulaeva, O.N., 2015. Effects of nitrate and ammonium on growth of arabidopsis thaliana plants transformed with the ARR5:: GUS construct and a role for cytokinins in suppression of disturbances induced by the presence of ammonium. J. Plant Physiol. 62 (6), 741–752.
Simmons, S.L., Bazylinski, D.A., Edwards, K.J., 2007. Population dynamics of marine magnetotactic bacteria in a meromictic salt pond described with qPCR. Environ. Microbiol. 9 (9), 2162–2174.
Tierney, J., Lars, A., Hedin, O., Wurzburger N., 2019. Nitrogen fixation does not balance fire-induced nitrogen losses in longleaf pine savannas. Ecology 100, 7 e02735. <https://dx.doi.org/https://doi.org/10.1002/ecy.2735>.
Valera, C.L., Alexander, M., 1961. Nutrition and physiology of denitrifying bacteria. Plant Soil 15, 268–280. https://doi.org/10.1007/BF01400460.
Vega, J.A., Fontúrbel, T., Merino, A., Fernández, C., Ferreiro, A., Jiménez, E., 2013. Testing the suitability of visual indicators of soil burn severity to reflect changes in soil chemical and microbial properties in pine stands and shrublands. Plant Soil 369, 73–91.
Velasco, A.G.V., Probanza, A., Mañero, F.J.G., Treviño, A.C., Moreno, J.M., Garcia, J.A. L., 2009. Effect of fire and retardant on soil microbial activity and functional diversity in a Mediterranean pasture. Geoderma 153, 186–193.
Voisin, A.S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (Pisum Sativum L.). Plant Soil 243, 1, 31–42. https://doi.org/10.1023/A:1019966207970.
Wendeborn, S., 2019. The chemistry, biology and modulation of ammonium nitrification in soil. Angew. Chem. Int. Ed. https://doi.org/10.1002/anie.201903014.
Zhang, M., Wang, W., Wang, D., Heenan, M., Xu, Z., 2018. Short-term responses of soil nitrogen mineralization, nitrification and denitrification to prescribed burning in a suburban forest ecosystem of subtropical Australia. Sci. Total Environ. 642, 879–886. https://doi.org/10.1016/j.scitotenv.2018.06.144.
Zhang, Z., Chen, H., Mu, X., Zhang, S., Pang, S., Ohore, O.E., 2020. Nitrate application decreased microbial biodiversity but stimulated denitrifiers in epiphytic biofilms on Ceratophyllum demersum. J. Environ. Manag. 1, 269–110814. https://doi.org/10.1016/j.jenvman.2020.110814.
Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M., Arcenegui, V., Mataix-Beneyto, J., 2009. Changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain. Appl. Soil Ecol. 42, 315–323.