agriculture

MDPI

Article

Effects of the Fertilizer Added with DMPP on Soil Nitrous Oxide Emissions and Microbial Functional Diversity

Anna Tedeschi [1], Anna De Marco [2], Franca Polimeno [3], Paul Di Tommasi [4], Giuseppe Maglione [3], Lucia Ottaiano [5], Carmen Arena [6], Vincenzo Magliulo [4] and Luca Vitale [4,*]

1 Research Division Portici, Institute of Biosciences and Bioresources (IBBR), National Research Council of Italy (CNR), Via Università 133, Portici, 80055 Naples, Italy; anna.tedeschi@cnr.it
2 Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy; ademarco@unina.it
3 Institute for Animal Production System in Mediterranean Environment (ISPAAM), National Research Council of Italy (CNR), P.le Enrico Ferni 1, Loc. Porto del Granatello, Portici, 80055 Naples, Italy; franca.polimeno@cnr.it (F.P.); giuseppe.maglione@cnr.it (G.M.)
4 Institute for Agricultural and Forestry Systems in the Mediterranean (ISAFoM), National Research Council of Italy (CNR), P.le Enrico Ferni 1, Loc. Porto del Granatello, Portici, 80055 Naples, Italy; paul.ditommasi@cnr.it (P.D.T.); enzo.magliulo@cnr.it (V.M.)
5 Department of Agricultural Science, University of Naples Federico II, via Università, 133, Portici, 80055 Naples, Italy; lucia.ottaiano@unina.it
6 Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy; c.arena@unina.it
* Correspondence: luca.vitale@cnr.it; Tel.: +36-06-499327526

Abstract: Agricultural sites contribute extensively to atmospheric emissions of climate-altering gases such as nitrous oxide. Several strategies have been considered to mitigate the impact of agriculture on climate, among these the utilization of fertilizers added with nitrification inhibitors such as DMPP (3,4-dimethylpyrazole phosphate) may represent a suitable solution. DMPP inhibits the growth and activity of ammonia-oxidizing microorganisms, particularly the ammonia-oxidizing bacteria, which are involved in N_2O production. At present, little information is available on the effects of DMPP on the catabolic diversity of soil microbial community. In this study, the N_2O emission by soil was performed by using the static chamber technique. The biological determinations of the microbial biomass carbon and the catabolic profile were assessed by measuring the substrate-induced respiration during the entire growing season of a potato crop under two nitrogen treatments: fertilization with and without DMPP. Our results did not show a clear mitigation of N_2O emission by DMPP, even if a tendency to lower N_2O fluxes in DMPP plots occurred when soil temperatures were lower than 20 °C. Conversely, DMPP deeply affected the microbial biomass and the catabolism of soil microorganisms, exerting a negative effect when it accumulated in excessive doses in the soil, limiting the growth and the capacity of soil microorganism communities to use different substrates.

Keywords: greenhouse gas emission; catabolic diversity; N fertilization; soil microbial communities

Citation: Tedeschi, A.; De Marco, A.; Polimeno, F.; Di Tommasi, P.; Maglione, G.; Ottaiano, L.; Arena, C.; Magliulo, V.; Vitale, L. Effects of the Fertilizer Added with DMPP on Soil Nitrous Oxide Emissions and Microbial Functional Diversity. *Agriculture* **2021**, *11*, 12. https://dx.doi.org/10.3390/agriculture11010012

Received: 15 October 2020
Accepted: 23 December 2020
Published: 27 December 2020

1. Introduction

Nitrogen (N) is an essential nutrient for plants and it is often the most limiting factor for crop production. Generally, nitrogen is applied to agricultural soils in a mineral or organic source, frequently in excess of the crop nutritional needs. Nitrogen is used not only by plants but also by soil microorganisms. Microbes are intimately involved in the biological and biochemical transformations occurring in the soil. They are responsible for the mineralization of organic matter and the release of nutrients absorbed by plants. Thus, microbes play an essential role in soil processes, significantly affecting the agroecosystem health through organic matter decomposition, nutrient cycling, and interaction with plants.

Some of N applied to the soil is lost as nitrate (NO_3^-) by leaching or gas emission, mainly nitrous oxide (N_2O), making agriculture a sector with a great impact on climate

and global environment [1]. The N loss as N_2O is due to nitrification and denitrification processes, which are controlled by soil physical–chemical conditions such as humidity, temperature, nitrogen and oxygen availability, and pH [2–6]. During nitrification occurring under aerobic conditions, ammonium nitrogen is oxidized to nitrite and nitrate, then reduced in the denitrification process under anaerobic conditions. The supply of ammonium-based fertilizers or organic fertilizers to soil determines an enrichment of ammonium in the soil, leading to the production of NO_3^- and N_2O [4,5].

Different strategies have been utilized in order to mitigate the emissions of climate-altering greenhouse gases (GHGs) from agricultural sites [7]. Since 1950, many molecules have been identified as possible nitrification inhibitors (NI), i.e., substances constraining N loss from agricultural soils. These compounds added to the fertilizer act to mitigate N loss as NO_3^- and N_2O by inhibiting biological NH_4^+ oxidation, and at the same time, increasing the nitrogen use efficiency (NUE) and yield of crops [8,9]. Among the NIs, 3,4-dimethylpyrazole phosphate (DMPP) has been proposed as an advantageous and practical tool for having several characteristics such as low application rate, high efficiency, and low ecotoxicity [10–12].

Most studies on DMPP focused attention on its efficiency in reducing soil N_2O emission [2,3,9,13] and its effects on soil microbial biomass and activity [14–16]. These researchers examined the abundance and structure of soil microbial communities in response to DMPP addition, particularly the ammonia-oxidizing microorganisms [15–20], but little information is available on the effects of DMPP on functional diversity of soil microbial communities. The microbial functional diversity relates to the capacity to perform different ecological processes in a soil and to utilize a wide collection of substrates. Direct measurements of functional diversity of soil microorganisms could provide more relevant information on soil functioning than measurements of species diversity or community structure, since microorganisms may be present in the soil, but often under rest or dormant phases and not functionally active [21].

In this study, we investigated (1) the effectiveness of DMPP in mitigating N_2O emission and (2) the outcomes of DMPP application to soil on microbial biomass, and more specifically, the development of the functional responses by soil microbial community after nitrogen fertilization.

2. Materials and Methods

2.1. Experimental Site

The study was carried out at the experimental farm of the Agricultural Technical Institute "De Cillis" in Ponticelli (Naples, Italy) during spring–summer 2013. The area is characterized by a typical Mediterranean climate with cold, rainy winters and hot, dry summers (Figure 1).

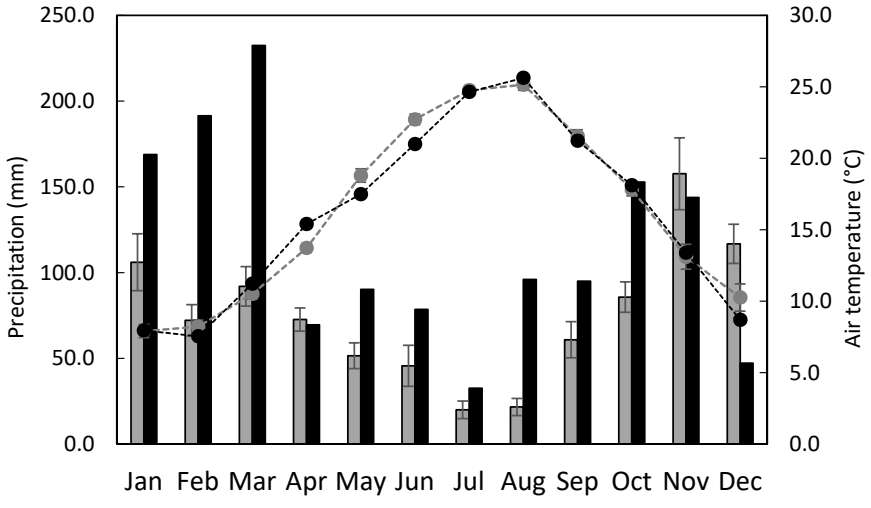

Figure 1. Monthly average air temperature (circles) and rainfall (bars). Gray: mean 1996–2012 values; black: 2013 values.

The soil has a coarse texture (loamy-sand according the USDA textural soil classification) being of volcanic origin. The main soil chemical–physical properties, determined before starting the experiment, are reported in Table S1. *Solanum tuberosum* L. (cv Agria) tubers were sown on 2013 April 08 on 3 m rows spaced by 0.30 m (3.0 × 3.0 m plot). The experiment was a randomized complete block design with two nitrogen treatments and three replicates. Two treatments were set-up: nitrogen fertilization without nitrification inhibitor (Nitrophoska perfect©, 15:5:20 N:K:P, N as 8% NH_4^+ and 7% NO_3^-, and 20% S as SO_3) (NH_4NO_3 plots) and nitrogen fertilization with nitrification inhibitor, the 3,5-dimethylpyrazole phosphate (DMPP) (Entec perfect©, 14:7:17 N:K:P, N as 7.9% NH_4^+ and 6.1% NO_3^-, and 22% S as SO_3; DMPP 0.8% of NH_4^+) (DMPP plots). Fertilizer was supplied on three events: when sown (100 kg N/ha), and on 04 June 2013 (30 kg N/ha) and 21 June 2013 (30 kg N/ha) by applying the fertilizer near hills that were made on 29 April 2013 by using a disc-type hiller. The amount of N per plot was distributed on the rows per plot. The resulting amount of DMPP applied was 13.9 μg/g dry soil and 4.2 μg/g dry soil, corresponding to the amount of 100 kg N/ha and 30 kg N/ha, respectively. The crop was well watered by means of a sprinkler to avoid water stress during growth.

2.2. Biometrical and Physiological Determinations

Harvesting occurred on 09 July. All plants inside each plot were harvested. Tubers were collected, whereas shoot biomass was dried in oven at 75 °C up to constant weight.

The chlorophyll content was used as a proxy to evaluate the plant greenness and was determined by a SPAD (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies Inc.) at three different times during the plant growing season. Two plants per plot were used and the measurements were performed on three leaves per plant (top, middle, and bottom leaves). Data for each plant were averaged.

2.3. Soil Temperature, Humidity, and Nitrogen Content

Soil temperature and moisture were measured during the whole growing season by means of a 107 probe (Campbell Scientific, Inc., Logan, UT, USA) and the CS616 reflectometer (Campbell Scientific, Inc., Logan, UT, USA), respectively. Both probes were introduced into the soil at a 0.0–0.05 m depth inside the chamber and connected to a datalogger (CR1000, Campbell Scientific, Inc., Logan, UT, USA). The water filled pore space (WFPS) was estimated as:

$$\text{WFPS} = \text{VSWC}/[1 - (\text{BD}/2.65)] \times 100$$

where VSWC represents the volumetric soil water content, 2.65 is the average density of the solid matrix calculated on the basis of the relative content of the different mineral constituents, and BD is the bulk density calculated as the dry weight of soil divided by its volume.

Total nitrogen (N_{tot}) content in the soil was determined by collecting samples from the 0.0–0.10 m soil layer. An integrated soil sample per plot was obtained by collecting and mixing together three different soil samples near each autochamber. Soil was air-dried and sieved (2 mm). Total nitrogen and carbon content was determined by gas chromatograph (Thermo Finnigan, CNS Analyzer).

2.4. Soil N_2O Emissions and Treatments with Acetylene (C_2H_2)

Soil N_2O emission measurements were performed four times during the day and for the whole growing season by automatic chambers (0.30 m diameter and 0.1 m height) linked through a Teflon tube to a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA, USA). One chamber per plot was positioned between rows and inserted at 0.03 m depth in the soil. Details on the protocol for gas and flux measurements have been reported in Tedeschi et al. [6].

The contribution of denitrification to total N_2O fluxes was evaluated by the acetylene inhibition technique (AIT). Soil samples were collected near autochambers at 0.0–0.10 m

depth by a PVC cylinder and put into a homemade PVC incubator [5]. The head-space of the incubator was filled with C_2H_2 to inhibit the nitrification and samples incubated for 6 h at ambient temperature [5]. Untreated samples were also prepared. The air space inside the incubator was frequently mixed by syringes. Air samples were collected at 3 h and 6 h from the head-space with a syringe and analyzed by an SRI 8610C gas chromatograph.

2.5. Soil Biological Analisys

Biological analysis were conducted on the same soil subsamples used for the determination of soil N content.

The microbial biomass carbon (C_{mic}) was evaluated by the substrate-induced respiration (SIR) method as CO_2 evolution from soil following the addition of 2 mL of a D-glucose solution (75 mM) and after the incubation in closed vials (30 mL) for 4 h at 25 °C in the darkness [22]. CO_2 efflux from each sample was measured by an infrared gas analyzer (Model LI6262, LI-COR, Lincoln, NE, USA).

The basal microbial respiration (R_{esp}) was measured through the LI6262 analyzer and determined after the incubation in vials for 4 h at 25 °C in darkness, following the addition of 2 mL of distilled water to the samples.

The soil metabolic quotient (qCO_2), which is the amount of CO_2-carbon produced per unit of microbial biomass carbon, was calculated as the ratio between basal respiration (R_{esp}) and C_{mic}. The soil metabolic quotient (qCO_2) is an index to evaluate the stress degree of the microbial biomass.

The catabolic response for different substrate types was also determined by measuring the short-term respiration [22] after incubation of the samples for 4 h at 25 °C in darkness following the addition of 19 substrates, namely 7 amino acids (AA), 2 carbohydrates (CH), and 10 carboxylic acids (CA).

The catabolic evenness (E) was determined as $E = 1/\sum p_i^2$, where p_i is the ratio of CO_2 evolved for a particular substrate to the sum of all substrates respired.

2.6. Statistical Analysis

Statistical analysis of data was performed by Sigma-Plot package (Sigma-Plot 12.2, Systat Software, Inc., San Jose, CA, USA). Differences in biometrical and physiological parameters between fertilization treatments were cheeked by paired t-test ($p \leq 0.05$), whereas differences between fertilization treatments or over time inside each treatment were cheeked by one-way ANOVA repeated measurements followed by Duncan's test ($p \leq 0.05$).

3. Results

3.1. Plant Greenness, Biomass, and Yield

The plant greenness was unaffected by the type of fertilizer applied (Table 1). A slight, but significant decrease of SPAD values was detected on 09 July, at the harvesting, compared to the previous records. The type of fertilizer did not influence the biomass production and the tuber yield (Table 1).

Table 1. SPAD values, abovegound biomass, and tuber yield in NH_4NO_3 plots and DMPP (3,4-dimethylpyrazole phosphate) plots. Data are means (n = 3) ± standard error.

Plot	SPAD			Aboveground Biomass (kg m^{-2})	Tuber Yield (kg m^{-2})
	06 June	20 June	09 July		
NH_4NO_3	47.64 ± 1.48 [a]	47.35 ± 1.68 [a]	43.429 ± 0.89 [b]	0.68 ± 0.068[a]	6.39 ± 0.26 [a]
DMPP	49.27 ± 1.76 [a]	51.64 ± 0.92 [a]	45.18 ± 1.94 [b]	0.59 ± 0.035[a]	5.78 ± 0.13 [a]

Different letters denote significant differences between treatments ($p \leq 0.05$). Data were analyzed by paired *t*-test.

3.2. Soil-Related Measurements and N_2O Emission

Soil pH showed variations during the studied period and ranged from 6 to 8 in both treatments (Figure 2a). The wider variation in soil pH was observed in DMPP plots, which dropped to 6 soon after the second fertilization event (Figure 2a); at that time, soil pH was lower in DMPP plots compared to NH_4NO_3 plots. Total nitrogen content was higher in DMPP plots compared to NH_4NO_3 plots after the first fertilization (Figure 2b). In response to the two additional fertilizations, the DMPP plots showed a lower N content compared to NH_4NO_3 plots, particularly at the end of the June (Figure 2b).

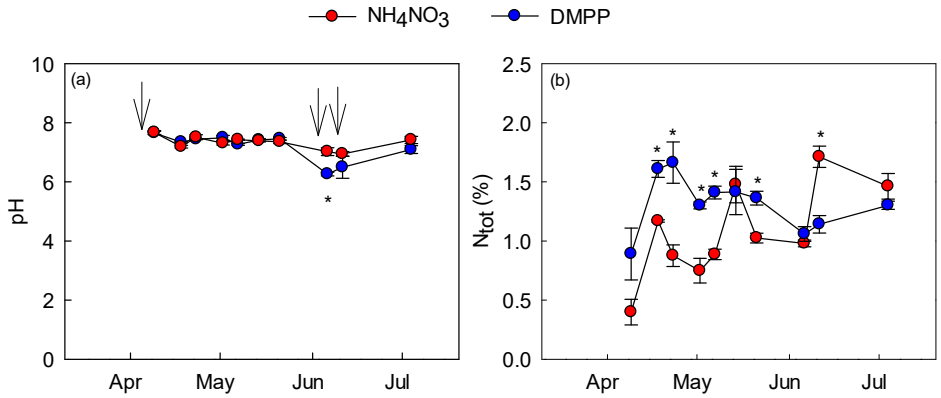

Figure 2. Soil pH (**a**) and total nitrogen (N_{tot}) content (**b**) in NH_4NO_3 and DMPP plots. Data are means (n = 3) ± standard error. Arrows show the fertilization events; asterisks indicate significant differences between treatments ($p \leq 0.05$). Data were analyzed by one-way ANOVA repeated measurements followed by Duncan's test.

Soil temperature increased with the progression of the experimental season (Figure 3a). The soil temperatures remained below 25 °C until significant volumes of irrigation water were supplied. Peaks higher than 30 °C occurred during the growth season, especially at the end of June (Figure 3a). The soil humidity, evaluated as water filled pore space (WFPS), changed during the season, following the irrigation events (Figure 3a). WFPS peaked after irrigation, ranging from 80% at the beginning of the experiment to 20% at the end of the plant growing season. In the remaining part of the study period, WFPS values were lower than 70% (Figure 3a).

Overall, the N_2O fluxes did not differ between the fertilization treatments (Figure 3b,c), even if a tendency to lower values in DMPP plots compared to NH_4NO_3 plots was observed after the first fertilization, when soil temperatures were about 20 °C. In particular, a peak of 80 μg m^{-2} h^{-1} was measured in the NH_4NO_3 plots (Figure 3b), whereas it was about 56 μg m^{-2} h^{-1} in DMPP plots (Figure 3c). Thereafter, in concomitance with soil temperature higher than 20 °C, N_2O fluxes measured in DMPP plots were higher—although not significant—compared to those measured in NH_4NO_3 plots (Figure 3b,c). For both treatments, peaks of variable amplitude in N_2O emission occurred in response to the irrigation that increased the soil humidity and, in turn, the WFPS values.

The acetylene inhibition technique was used to evaluate the denitrification contribution to total N_2O flux. Based on this method, the addition of C_2H_2, as a nitrification inhibitor, to soil cores collected in the field and stored for a short-term in PVC incubators, gave values approaching to zero (Figure 3b,c).

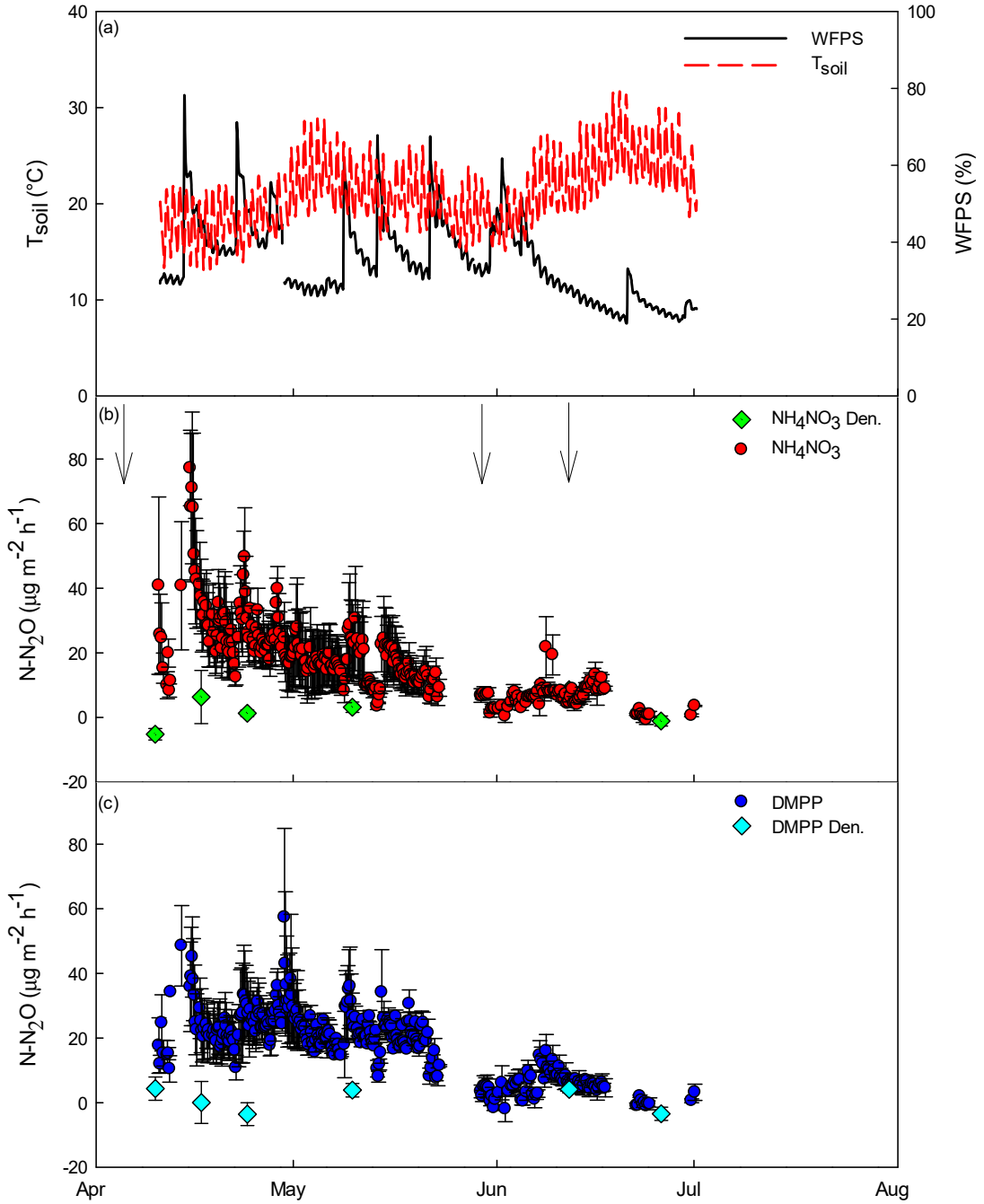

Figure 3. Soil average temperature (Tsoil) and water filled pore space WFPS (**a**) and nitrous oxide fluxes in NH_4NO_3 (**b**) and DMPP plots (**c**). Data are means (n = 3) ± standard error. Arrows show the fertilization events. The circles show the total N-N_2O fluxes; rhombuses show N-N_2O fluxes due to the denitrification. Data were analyzed by one-way ANOVA repeated measurements followed by Duncan's test.

3.3. Soil Microbial Activity

The microbial biomass carbon (C_{mic}) was negatively affected by the first fertilization event occurring when sown in response to high N rates (100 Kg ha^{-1}). The C_{mic} reduction in NH_3NO_3 plots was lower compared to that observed in DMPP plots (Figure 4a). During the potato vegetative growth, corresponding to the middle experimental period, an increase of microbial biomass in DMPP plots was detected. Conversely, C_{mic} measured in NH_4NO_3 plots decreased over time until further fertilizations. Following the two fertilization appli-

cations, provided at a rate of 30 kg N ha^{-1}, C_{mic} showed a growing trend in both nitrogen treatments. At harvest and at the beginning of the experiment, C_{mic} showed comparable values (Figure 4a).

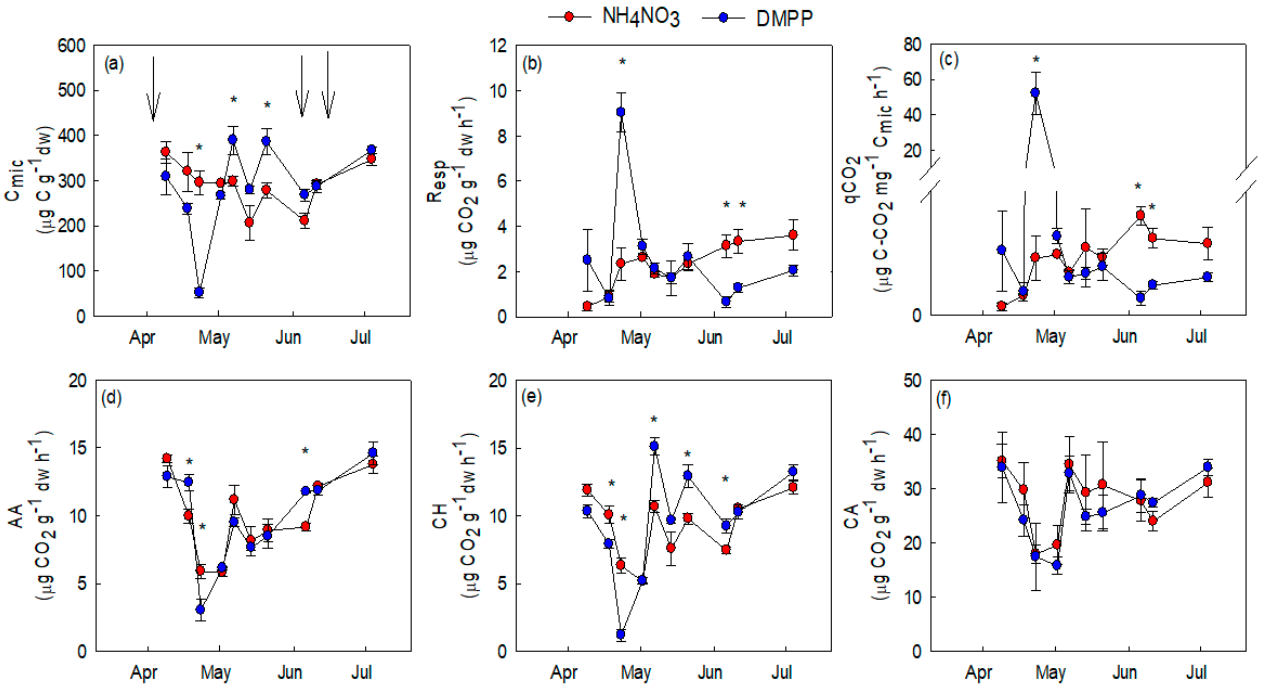

Figure 4. Microbial biomass carbon (Cmic) (**a**), basal microbial respiration (Resp) (**b**), metabolic quotient, qCO_2 (**c**), catabolic response to aminoacids (AA) (**d**), catabolic response to carbohydrates (CH) (**e**), and catabolic response to carboxylic acids (CA) (**f**), in NH_4NO_3 and DMPP plots. Data are means (n = 3) ± standard error. Arrows show the fertilization events, asterisks indicate significant difference between treatments ($p \leq 0.05$). Data were analyzed by one-way ANOVA repeated measurements followed by Duncan's test.

The basal microbial respiration (R_{esp}) increased in NH_3NO_3 plots after the first fertilization (Figure 4b) and showed a peak in DMPP plots on the 20th day. Afterward, compared to the initial values, R_{esp} increased in NH_3NO_3 plots, reaching the highest value at the end of the experiment, while it decreased in DMPP plots. In response to the two following fertilizations, NH_3NO_3 plots showed higher R_{esp} values compared to DMPP plots.

The soil metabolic quotient qCO_2 mirrored the trend of microbial respiration (Figure 4c) in both plots, showing the highest value of about 60 µg C-CO_2 mg^{-1} C_{mic} h^{-1} in DMPP plots in combination with the highest basal respiration and the lowest microbial biomass carbon.

The catabolic response profile was also deeply affected by the first fertilization event (Figure 4d–f). The substrates-induced respiration decrease in both N treatments; however, the reduction was more marked on the third sampling date in DMPP plots compared to NH_4NO_3 plots, especially for the catabolic response to AA and CH substrates. A similar trend was also observed for the catabolic response to CA substrates. With the progression of the vegetative growing season, the catabolic response reached the initial values, with no particular differences in trends between the two treatments, except for the CH substrates, which showed higher CO_2 production in DMPP plots compared to NH_4NO_3 plots.

The catabolic evenness did not show appreciable differences either over time or between treatments (Figure 5). A significant reduction in catabolic evenness on the third sampling date in DMPP plots compared to NH_4NO_3 plots was evident, the marked alterations occurring together in microbial biomass and respiration.

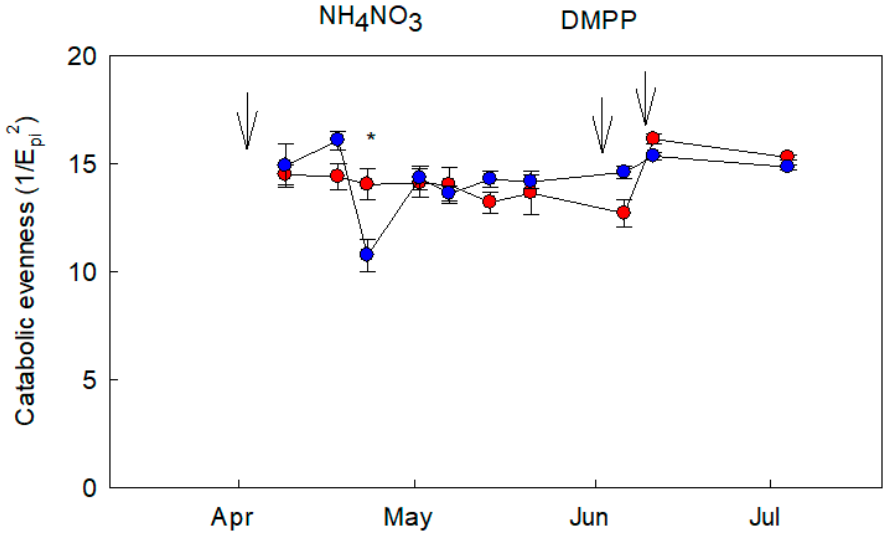

Figure 5. Catabolic evenness in NH_4NO_3 and DMPP plots. Data are means (n = 3) ± standard error. Arrows show the fertilization events, asterisks indicate significant difference between treatments ($p \leq 0.05$). Data were analyzed by one-way ANOVA repeated measurements followed by Duncan's test.

4. Discussion

Agriculture is one of the main sectors contributing to environmental alteration, particularly by climate change through the farm management practices. In order to reduce the negative impact of agriculture on climate and environment, different strategies have been proposed and applied in the last few decades. Among these, enhanced fertilizer use efficiency by crop is a promising tool for mitigating climate-altering gas emissions [23]. Better fertilizer use efficiency may be achieved through the development of fertilizers added with specific inhibitors of biological transformations occurring in the soil, particularly nitrification [4].

In this study, we used 3,4-dimethylpyrazole phosphate (DMPP) as a nitrification inhibitor. Many experimental evidences demonstrated how this specific compound is effective in improving fertilizer use efficiency by crop and in reducing N_2O emissions from agricultural soils [14–16]. Our data clearly showed that the application of DMPP to the fertilizer improved the soil nitrogen content. Indeed, it is likely that DMPP inhibited ammonia oxidation [16], and WFPS values did not favor nitrate leaching. However, DMPP application did not cause a marked mitigation of N_2O emission compared to the NH_4NO_3 plots, even if a slight decrease of N_2O fluxes was observed immediately after the first fertilization application. In these circumstances, the WFPS values were high and the soil temperature was lower than 20 °C, which might represent the threshold above which the effectiveness of DMPP was progressively reduced [3,24]. From May and beyond, greater N_2O fluxes were measured in DMPP plots compared to NH_4NO_3 plots, and no improvement in physiological traits (SPAD and aboveground biomass) or in agronomical traits (tuber yield) was found, confirming the loss of DMPP effectiveness with increasing soil temperatures.

The nitrous oxide emissions were mainly produced by nitrification, as demonstrated by flows close to zero in correspondence with the treatments with acetylene (which completely inhibited nitrification). Moreover, the soil water filled pore space was below 70%—a watershed value between nitrification and denitrification—for most of the period studied [25], even if the peaks in N_2O emission after the first fertilization event were due to a rise in WFPS above 70%. Many evidences have confirmed the functional importance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in N_2O production during the nitrification [26–28]. We suppose that under our experimental conditions AOB were likely the main contributors to N_2O production, since AOA gain advantage from

growth on acidic and NH_4^+-poor soils, while our experimental site showed an alkaline or subalkaline pH, for most of the study phase. However, the contribution of the heterotrophic denitrification in producing N_2O during the experimental period, especially for WFPS between 50–70%, cannot be excluded, even if it seems to have a negligible role compared to nitrification, which is unexpected for WFPS values greater than 70%. Barrera et al. [29] found that DMPP induced the abundance of *nosZ* gene—codifying for the nitrous oxide reductase—diminishing N_2O losses at high water content below 80%. This hypothesis could also explain the lower N_2O peak observed in DMPP plots compared to NH_4NO_3 plots for WFPS values higher than 70%.

Most of studies on the effects of DMPP on soil microorganisms have been aimed at understanding the impacts of DMPP on nitrification and dynamics of ammonia oxidizers [17–20], whereas little information is available on the impact of DMPP on the functional diversity of the soil microbial communities. It is well known that microbial functional diversity represents the capacity to perform different ecological processes and to utilize a wide assortment of substrates. In this study, we used the substrate-induced respiration (SIR) technique to assess the microbial functional diversity, and the catabolic evenness (E) as an indicator to evaluate the variability in substrate utilization by soil microbial communities. The N fertilization per se negatively affected, at least at higher fertilizer application rates, the physiological responses of microbial communities, determining a reduction in the microbial biomass carbon (C_{mic}) and community functional diversity. Our data agree with some studies reporting a decrease in microbial biomass with N addition [30,31]. Moreover, C_{mic} showed a negative response to high fertilizer application rates, likely because the microbial communities become C limited, resulting from nitrogen accumulation in the soil, due to the absence of plants in this phase and, therefore, in nutrient uptake. This effect was more evident in DMPP plots where the reduction in C_{mic} was higher than in NH_4NO_3 plots, followed by an opposite trend in N content. However, it is improbable that only N accumulation may induce stress conditions for the soil microbial community. A negative effect of the 3,4-dimethylpyrazole phosphate on microbial communities cannot be excluded on the basis of the drastic reduction in microbial biomass followed by the increase of microbial respiration and qCO_2 on the third sampling data, even if previous reports showed no negative effect of DMPP on nontarget microorganisms in soils when NI was applied at agronomic doses [32,33]. The increase in microbial respiration and in qCO_2 found in DMPP plots indicated a stress condition for microbial communities, probably related to DMPP in the soil. The method of distributing fertilizer adopted in this study resulted in large DMPP doses in the soil, about 14 μg/g dry soil, after the first fertilization, which may have inhibited the growth and activity of microbes. Tindaon et al. [32] reported inhibitory effects of DMPP on nontarget microorganisms at doses greater than 5 μg/g dry soil in light-textured sandy soil. We hypothesize that the local accumulation of large DMPP doses in the soil may have also constrained the activity of nontarget microorganisms. On the other hand, the application of fertilizer at lower rates (30 kg N ha^{-1}), corresponding to about 4.2 μg/g dry soil. DMPP, occurred later during the growing season and did not involve a negative response by microorganisms.

DMPP would seem to have adverse effects on ammonia oxidizer and nontarget soil microorganism populations, as well as on their functions. The functional diversity of soil microbial community was deeply affected by the high DMPP doses, as indicated by the catabolic response profile. The soil amended with DMPP showed a reduced biological activity compared to soil amended with fertilizer without nitrification inhibitor, the former being characterized by a reduced evenness (E). In these soils, microbial communities exhibited a reduced capacity to utilize a wide spectrum of C sources and seemed more adapted to consume only some simple carbon compounds, such as carboxylic acids. It is likely to suppose that high doses of DMPP caused alteration in the composition of soil microbial communities, highlighted by a reduction in the catabolic response, favoring some microbial populations more than others. The loss of DMPP effects with the time and under soil temperature higher than 20 °C enhanced the carbon microbial biomass and improved

the functional diversity of soil microbial communities, emphasizing the inhibitory effect of 3,4-dimethylpyrazole phosphate on microbial communities when this NI is accumulated in the soil at high doses.

5. Conclusions

In the present study, the application of fertilizer added with DMPP did not clearly promote mitigation of N_2O emission, even if a tendency to lower N_2O fluxes in DMPP plots occurred when soil temperatures were lower than 20 °C. DMPP would seem to inhibit the growth and metabolism of soil microorganisms. The microbial functional diversity is deeply and negatively affected by DMPP when it is accumulated in excessive doses in the soil, thus limiting the growth and the capacity of soil microorganism communities to utilize different substrates. Our study may provide useful information about the correct application of fertilizer with added DMPP nitrification inhibitor. However, data refer to a single agronomical year and further studies are needed to confirm these promising results.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0472/11/1/12/s1, Table S1: Soil chemical–physical characteristics.

Author Contributions: Conceptualization, V.M. and L.V.; investigation, A.T., A.D.M., L.O., G.M., P.D.T., C.A., and L.V.; methodology, A.D.M., P.D.T., and L.V.; data curation, A.T., A.D.M., F.P., C.A., and L.V.; writing—original draft preparation, L.V.; writing—review and editing, A.T., A.D.M., C.A., V.M, and L.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the ISAFoM-CNR and Department of Biology of University of Naples Federico II.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Authors thank M. Tosca, B. Di Matteo, and F. Di Matteo for their valuable contribution to the experimental campaign. Thanks are due to D. Calandrelli for meteorological data. Authors thank K + S Agricoltura S.p.a. (Cesano Modeno MB, Italy) for the supply of Entec© Fertilizer and Dante Francesco Giovine (K + S Nitrogen, Cesano Modeno, Italy) for his support to crop management.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. Climate Smart Agriculture Sourcebook. Available online: http://www.fao.org/climate-smart-agriculture-sourcebook/production-resources/module-b1-crops/chapter-b1-1 (accessed on 26 December 2020).
2. Vitale, L.; Polimeno, F.; Ottaiano, L.; Maglione, G.; Tedeschi, A.; De Marco, A.; Di Tommasi, P.; Mori, M.; Magliulo, V. Fertilizer type influences tomato yield and soil N_2O emissions. *Plant. Soil Environ.* **2017**, *63*, 105–110.
3. Vitale, L.; Tedeschi, A.; Polimeno, F.; Ottaiano, L.; Maglione, G.; Arena, C.; De Marco, A.; Magliulo, V. Water regime affects soil N_2O emission and tomato yield grown under different types of fertilisers. *Ital. J. Agron.* **2018**, *13*, 74–79. [CrossRef]
4. Norton, J.; Ouyang, Y. Controls and adaptive management of nitrification in agricultural soils. *Front. Microbiol.* **2019**, *10*, 1931. [CrossRef]
5. Forte, A.; Fierro, A. Denitrification rate and its potential to predict biogenic N_2O field emissions in a Mediterranean maize-cropped soil in Southern Italy. *Land* **2019**, *8*, 97. [CrossRef]
6. Tedeschi, A.; Volpe, M.G.; Polimeno, F.; Siano, F.; Maglione, G.; Di Tommasi, P.; Vasca, E.; Magliulo, V.; Vitale, L. Soil fertilization with urea has little effect on seed quality but reduces soil N_2O emissions from a hemp cultivation. *Agriculture* **2020**, *10*, 240. [CrossRef]
7. Smith, P.; Martino, D.; Cai, Z.; Gwary, D.; Janzen, H.; Kumar, P.; McCarl, B.; Ogle, S.; O'Mara, F.; Rice, C.; et al. Agriculture. In *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., Meyer, L.A., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2007; pp. 497–540.
8. Cassman, K.G.; Dobermann, A.R.; Walters, D. Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio* **2002**, *31*, 132–140. [CrossRef]
9. Herr, C.; Mannheim, T.; Müller, T.; Ruser, R. Effect of nitrification inhibitors on N_2O emissions after cattle slurry application. *Agronomy* **2020**, *10*, 1174. [CrossRef]

10. Zerulla, W.; Barth, T.; Dressel, J.; Erhardt, K.; von Locquenghien, K.H.; Pasda, G.; Rädle, M.; Wissemeier, A. 3,4-dimethylpyrazole phosphate (DMPP)—A new nitrification inhibitor for agriculture and horticulture. *Biol. Fertil. Soils* **2001**, *34*, 79–84. [CrossRef]
11. Weiske, A.; Benckiser, G.; Herbert, T.; Ottow, J. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. *Biol. Fertil. Soils* **2001**, *34*, 109–117.
12. Zerulla, W.; Pasda, G.; Hähndel, R.; Wissemeier, A. The new nitrification inhibitor DMPP (ENTEC®) for use in agricultural and horticultural crops—An overview. *Plant Nutr.* **2001**, *92*, 754–755.
13. Recio, J.; Vallejo, A.; Le-Noë, J.; Garnier, J.; García-Marco, S.; Álvarez, J.M.; Sanz-Cobena, A. The effect of nitrification inhibitors on NH3 and N2O emissions in highly N fertilized irrigated Mediterranean cropping systems. *Sci. Total Environ.* **2018**, *636*, 427–436. [CrossRef] [PubMed]
14. Friedl, J.; Scheer, C.; Rowlings, D.W.; Deltedesco, E.; Gorfer, M.; De Rosa, D.; Grace, P.R.; Müller, C.; Keiblinge, K.M. Efect of the nitrifcation inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on N-turnover, the N_2O reductase-gene nosZ and N_2O:N_2 partitioning from agricultural soils. *Sci. Rep.* **2020**, *10*, 2399. [CrossRef] [PubMed]
15. Fuertes-Mendizábal, T.; Huérfano, X.; Vega-Mas, I.; Torralbo, F.; Menéndez, S.; Ippolito, J.A.; Kammann, C.; Wrage-Mönnig, N.; Cayuela, M.L.; Borchard, N.; et al. Biochar reduces the efficiency of nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) mitigating N_2O emissions. *Sci. Rep.* **2019**, *9*, 2346. [CrossRef]
16. Duan, Y.F.; Kong, X.W.; Schramm, A.; Labouriau, R.; Eriksen, J.; Petersen, S.O. Microbial N Transformations and N_2O Emission after Simulated Grassland Cultivation: Effects of the Nitrification Inhibitor 3,4-Dimethylpyrazole Phosphate (DMPP). *Appl. Environ. Microbiol.* **2017**, *83*, e02019-16. [CrossRef]
17. Shi, X.; Hu, H.W.; Müller, C.; He, J.Z.; Chen, D.; Suter, H.C. Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrification and nitrifiers in two contrasting agricultural soils. *Appl. Environ. Microbiol.* **2016**, *82*, 5236–5248. [CrossRef]
18. Benckiser, G.; Christ, E.; Herbert, T.; Weiske, A.; Blome, J.; Hardt, M. The nitrification inhibitor 3,4 dimethylpyrazole-phosphat (DMPP)—Quantification and effects on soil metabolism. *Plant Soil* **2013**, *371*, 257–266. [CrossRef]
19. Dong, X.X.; Zhang, L.L.; Wu, Z.J.; Zhang, H.W.; Gong, P. The response of nitrifier, N-fixer and denitrifier gene copy numbers to the nitrification inhibitor 3,4-dimethylpyrazole phosphate. *Plant Soil Environ.* **2013**, *59*, 398–403.
20. Liu, R.; Hayden, H.; Suter, H.; He, J.; Chen, D. The effect of nitrification inhibitors in reducing nitrification and the ammonia oxidizer population in three contrasting soils. *J. Soils Sediments* **2015**, *15*, 1113–1118. [CrossRef]
21. White, D.C.; MacNaughton, S.J. Chemical and molecular approaches for rapid assessment of the biological status of soils. In *Biological Indicators of Soil Health*; Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., Eds.; CAB: Wallingford, CO, USA, 1997; pp. 371–396.
22. Degens, B.P.; Schipper, L.A.; Sparling, G.P.; Duncan, L.C. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? *Soil Biol. Biochem.* **2001**, *33*, 1143–1153. [CrossRef]
23. Ventorino, V.; De Marco, A.; Pepe, O.; Virzo De Santo, A.; Moschetti, G. Impact of innovative agricultural practices of carbon sequestration on soil microbial community. In *Carbon Sequestration in Agricultural Soils. A Multidisciplinary Approach to Innovative Methods*; Piccolo, A., Ed.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 145–177.
24. Irigoyen, I.; Muro, J.; Azpilikueta, M.; Aparicio-Tejo, P.; Lamsfus, C. Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures. *Aust. J. Soil Res.* **2003**, *41*, 1177–1183. [CrossRef]
25. Machefert, S.E.; Dise, N.B. Hydrological controls on denitrification in riparian ecosystems. *Hydrol. Earth Syst. Sci.* **2004**, *8*, 686–694. [CrossRef]
26. Shen, J.P.; Zhang, L.M.; Hong, J.; Di, H.J.; He, J.Z. A review of ammonia-oxidizing bacteria and archaea in Chinese soils. *Front. Microbiol.* **2012**, *3*, 296. [CrossRef] [PubMed]
27. Wang, J.; Wang, W.D.; Gu, J.D. Community structure and abundance of ammonia-oxidizing archaea and bacteria after conversion from soybean to rice paddy in albic soils of Northeast China. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2765–2778. [CrossRef] [PubMed]
28. Zhao, D.Y.; Luo, J.; Wang, J.Q.; Huang, R.; Guo, K.; Li, Y.; Wu, Q.L. The influence of land use on the abundance and diversity of ammonia oxidizers. *Curr. Microbiol.* **2015**, *70*, 282–289. [CrossRef] [PubMed]
29. Barrena, I.; Menendez, S.; Correa-Galeote, D.; Vega-Mas, I.; Bedmar, E.J.; Gonzalez-Murua, C.; Estavillo, J.M. Soil water content modulates the effect of the nitrification inhibitor 3,4-Dimethylpyrazole Phosphate (DMPP) on nitrifying and denitrifying bacteria. *Geoderma* **2017**, *303*, 1–8. [CrossRef]
30. He, Y.; Qi, Y.; Dong, Y.; Xiao, S.; Peng, Q.; Liu, X.; Sun, L. Effects of nitrogenf on soil microbial biomass and community functional diversity in temperate grassland in inner Mongolia, China. *Clean Soil Air Water* **2013**, *41*, 1216–1221. [CrossRef]
31. Wang, C.; Liu, D.; Bai, E. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biol. Biochem.* **2018**, *120*, 126–133. [CrossRef]
32. Tindaon, F.; Benckiser, G.; Ottow, J.C.G. Evaluation of ecological doses of the nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) and 4-chloromethylpyrazole (ClMP) in comparison to dicyandiamide (DCD) in their effects on dehydrogenase and dimethyl sulfoxide reductase activity in soils. *Biol. Fertil. Soils* **2012**, *48*, 643–650. [CrossRef]
33. Kong, X.; Duan, Y.; Schramm, A.; Eriksen, J.; Petersen, S.O. 3,4-Dimethylpyrazole phosphate (DMPP) reduces activity of ammonia oxidizers without adverse effects on non-target soil microorganisms and functions. *Appl. Soil Ecol.* **2016**, *105*, 67–75. [CrossRef]