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Fungicide and Bactericide Effects on Carbon and Nitrogen Cycling in Soils: A Meta-Analysis

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Abstract: Fungi and bacteria play a central role in the cycling of carbon (C) and nitrogen (N), which has been frequently assessed by manipulating their abundance in soil with the application of fungicides and bactericides. We conducted a meta-analysis using 61 publications to investigate whether fungicides and bactericides have distinct effects on soil C- and N- cycling, and how they vary with land type and soil properties. Most fungicides and bactericides had significant negative effects on microbial biomass C and N. However, they had mixed effects on soil respiration, N pools, and transformation processes, varying strongly with the type of fungicide and bactericide. Available NO_3^- was lightly affected, while N_2O emission was reduced by most biocides. The application of fungicides had neutral effects on respiration, NH_4^+, and ammonification in agro-ecosystems, but positive effects in forests. Effect sizes of available NO_3^- and nitrification in response to bactericides were sensitive to soil pH and C content. Our results suggest that most fungicides and bactericides inhibit microbial growth, but that they have mixed effects on respiration and N cycling. Biocides need to be carefully evaluated for unintentional side effects before they are used in assessing the role of fungi and bacteria for C- and N- cycling.

Keywords: fungicide; bactericide; carbon and nitrogen cycle; land type

1. Introduction

Soil microbial communities play a central role in the breakdown of organic matter and cycling of carbon (C) and nutrients, and thereby play a significant role in greenhouse gas emission, soil C sequestration, and nutrient transformations [1,2]. The major groups in the soil are fungi and bacteria, while in many soils archaea are also important [3]. These microbial groups are susceptible to changes in environmental conditions, substrate availability, and land use change [4,5], with subsequent consequences for soil C and nutrient dynamics [6].

The soil microbial biomass is mostly comprised of fungi and bacteria, and both contribute to soil organic C storage [1,7] and cycling of nutrients in soil [8]. Indeed, much of the soil C originates from microbial biomass and large amounts of microbial residues can be stabilized in organo-mineral complexes [9–11] or within soil aggregates [12]. Studying the dynamics of fungi and bacteria may, therefore, help to understand the long-term soil C storage potential [7]. Although both fungi and bacteria are engaged in mineralization of C and nutrients in the soil, they differ in their demand for C and nutrients, morphology, physiology, and C use efficiency [13]. Fungi tend to be more important in decomposing soil organic matter with low nutrient content and poor quality because their nutrient demands and metabolic activities are low [14,15]. Bacteria contribute to the C cycling through decomposition of fresh dead plant biomass and are also important for the decomposition of dead fungal mycelia [16]. Fungi may store more C than bacteria because they tend to have higher C assimilation efficiencies [17]. On the other hand, fungi showed slower C turnover rates than bacteria [13,18].

Therefore, the relative abundance of fungi and bacteria, the dominant decomposers in soil, can have different influences on C cycling [7,19].

Nitrogen (N) transformations in soil, including ammonification, nitrification, and denitrification, are all mediated by microbes [20]. The relative abundance of fungi and bacteria can have significant impacts on these N transformation rates [21]. For instance, heterotrophic fungi and autotrophic bacteria both contributed significantly to nitrification in acidic forest soils [22]. However, while both fungi and bacteria can assimilate nitrate (NO_3^-) [23,24], heterotrophic bacteria preferred NO_3^- when fungi scavenged more effectively for ammonium (NH_4^+) in sagebrush–crested wheatgrass [25]. Low net N mineralization rates tend to occur in fungal-dominated soils with low biological activity [2,26] and extensive fungal hyphal networks [27]. On the other hand, high biological activity and net N mineralization rates tend to occur in bacteria-dominated soils [2,26]. Soils vary in their fungal:bacterial ratios where bacteria tend to prefer mostly fertile and N-rich soils [28], while fungi can tolerate low soil N concentrations [19,29]. These variations may help to understand their significant contributions to the soil N cycle.

The relative role of fungi and bacteria for soil C and N dynamics can be assessed by manipulating their abundance in soil with the application of fungicides and bactericides. The application of fungicides and bactericides to soil has become one of the most common techniques to manipulate microbial community composition [30], while they are frequently used in agriculture to control plant pathogens and soil-borne diseases [31]. Group-specific biocides can control the abundance and activity of the major microbial communities. Several studies found that different biocides have different impacts on total microbial communities, affecting both targeted and non-target organisms [32,33], depending on the amount applied [34–38]. Frequent exposure to biocides can result in microbial communities reducing soil fertility in agricultural systems and affecting other ecosystem properties [39]. Therefore, quantitative information regarding biocide use is urgently needed for facilitating their effective uses in experimental and ecological studies manipulating microbial communities.

We conducted a meta-analysis to understand the impact of different biocides on soil microbial biomass, and C and N cycling. We asked the following questions: (i) How do different biocides affect microbial biomass and C and N cycling in soil? (ii) How do the effects of fungicides and bactericides vary with land type? (iii) How do the effects of fungicides and bactericides vary with soil pH or with soil C content (indices of soil fertility)?

2. Materials and Methods

2.1. Data Compilation

For our meta-analysis, we collected data from publications that we searched in Scopus. We selected 18 keywords related to soil and microbial properties and processes (Table S1). For studies to be included in our data set we used the following criteria:

- Studies need to report soil and microbial properties for at least one biocide application and for a control.
- Studies need to report at least one of the following properties: Microbial biomass C and N, respiration rate, available NH_4^+, ammonification, available NO_3^-, nitrification, and N_2O.
- Studies need to report the amount of biocide applied, and the time of measurements since application.

We included studies where biocides were applied as solids or in solution for uniform distribution in soil. We found a total of 61 publications (References S1) and a total of 3026 observations (Supplementary Data). The number of publications of the selected papers increased with time (Figure S1). Biocides applications by country are shown in Table S2.

For each publication, we further noted location, land use, soil C content, and pH, when reported (Supplementary Data). Data in the original figures were extracted using Getdata Graph Digitizer

(version 2.22). Mean values (control and treatment), standard deviations, and the number of replicates was collected, when reported.

2.2. Statistical Analyses

For effect size calculation, we calculated the natural log of the response ratio lnRR = ln (mean treatment value/mean control value) [40]. The random-effects model in MetaWin 2.1 [41] was used to calculate the mean effect sizes and 95% bootstrapped confidence intervals (CI, 4999 iterations considered for bootstrapping). We compared the effects of biocide type by target group (fungicide, bactericide, fungicide, and bactericide combined) and for each individual biocide type. We adjusted the weight of each observation within a specific study by the number of observations so that studies with a large number of observations did not have a dominating effect on the mean effect size [42]. Usually, effect sizes are weighted by the inverse of the pooled variance calculated from standard deviations of the control and treatment [43]. However, because standard deviations were often not reported (Supplementary Data), we decided not to use this variance-based weighting, because it would exclude those observations where standard deviations were not reported in our meta-analysis. Others have shown that variance-based weighting of effect sizes gave similar results to no weighting [44,45]. We used $Q_{between}$ (heterogeneity in effect sizes associated to differences between categories) to test if effects differed among biocide types, between fungicides and bactericides, and among land types for fungicides and bactericides separately. Differences between categories were considered significant at $P_{random} < 0.05$. We then related the lnRR for the different parameters to biocide incubation time (day), soil pH and C (%), and biocide amount (mg g^{-1}) using linear regressions. We tested these relationships for fungicides and bactericides separately, and whether the slope of the relationship differed between fungicides and bactericides. For these relationships, we used JMP (version 8, SAS Institute, Cary, NC, USA). Relationships and differences in slopes were considered significant at $P < 0.05$.

3. Results

We examined a total of 35 fungicides and 3 bactericides (Table S3). These fungicides and bactericides differed in their molecular masses (ranging between 73 g mol^{-1} for lime-sulfur (F) to 581 g mol^{-1} for streptomycin (B)), C content (ranging between 0% for Cu oxychloride (F) and lime-sulfur (F) to 77% for tridemorph (F)), and N content (ranging between 0% for a number of biocides to 22% for carbendazim (F)).

The mean effect sizes (natural log of the response ratio, lnRR) of microbial biomass carbon (MBC) were not significantly different between fungicides and bactericides (P = 0.27, Figure 1a), but were found to be significantly different among the different biocide types (P = 0.0002, Figure 1b). Almost all biocides had negative effect sizes, except for the bactericide streptomycin, which had no effect on MBC. The highest negative response for MBC was observed for the fungicide tebuconazole (avg. lnRR = −1.62, n = 12, Figure 1b). Effect sizes of microbial biomass nitrogen (MBN) were also not significantly different between fungicides and bactericides (P = 0.32, Figure 1c), but significantly different among biocides (P = 0.0004), and mostly negative, except for benomyl (F), which showed a positive effect (Figure 1d). Cycloheximide (F) and streptomycin (B), as single applications or combined, showed similar responses for MBC and MBN (Figure 1b,d), where negative effects were observed for cycloheximide with and without streptomycin, while streptomycin alone had no effect. This indicates that the fungicide was more effective than the bactericide in reducing both MBC and MBN.

Soil respiration was not significantly different between fungicides and bactericides (P = 0.85, Figure 2a). However, biocide types showed significant different effect sizes for soil respiration (P = 0.0002), where no positive and negative effects on soil respiration were observed (Figure 2b). The most negative and positive effect sizes were found for Cu oxychloride (F, only four observations) and pentachlorophenol (F, only six observations), respectively. The fungicide propiconazole had no effect on respiration for 136 observations. Cycloheximide (F) and streptomycin (B) also had no effect on respiration either as single or mixed applications. On the other hand, captan (F) and bronopol (B)

both in single and combined doses had negative effects on soil respiration (Figure 2b). The bactericide oxytetracycline had a negative effect on respiration, and moreover, it had a negative effect on respiration when combined with the fungicide captan.

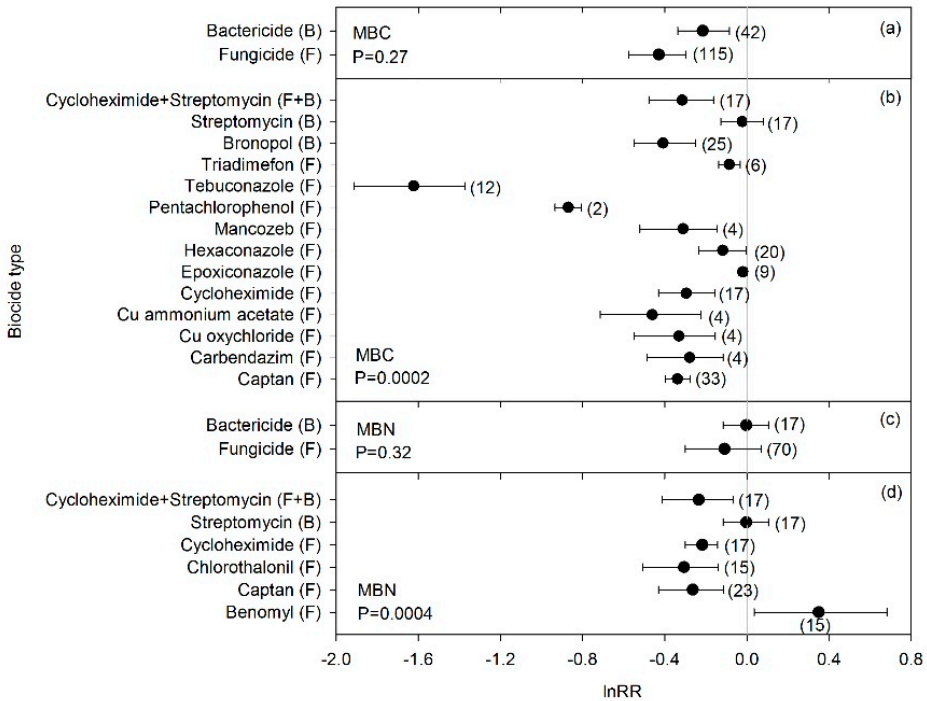

Figure 1. The mean response ratios (lnRR) of (**a**,**b**) microbial biomass carbon (MBC) and (**c**,**d**) microbial biomass nitrogen (MBN) to different biocide types (F: Fungicide, B: Bactericide, F+B: Fungicide + Bactericide). The error bars represent 95% bootstrapped confidence intervals (CIs). The effects of different biocides were considered significant if the 95% CI of the mean effect size did not overlap with zero. The numbers of observations for each biocide are shown next to the error bars in brackets.

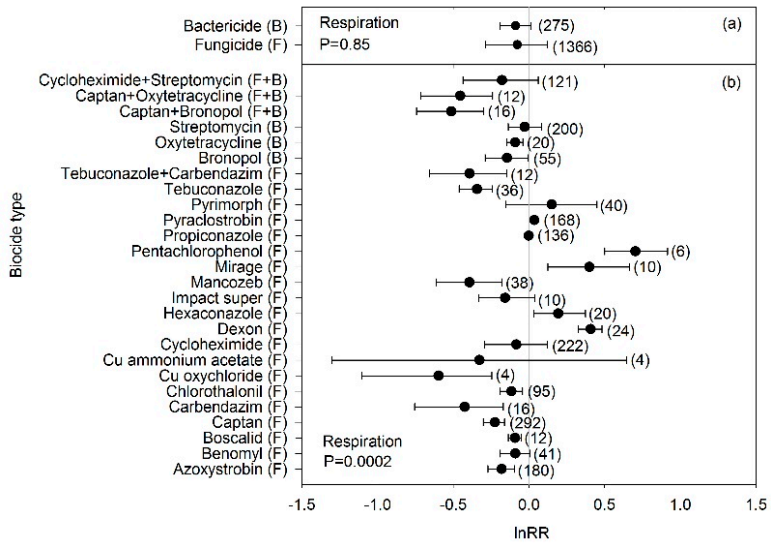

Figure 2. The mean response ratios (lnRR) of (**a**,**b**) soil respiration to different biocide types (F: Fungicide, B: Bactericide, F+B: Fungicide + Bactericide). The error bars represent 95% bootstrapped confidence intervals (CIs). The effects of different biocides were considered significant if the 95% CI of the mean effect size did not overlap with zero. The numbers of observations for each biocide are shown next to the error bars in brackets.

Soil available ammonium (NH_4^+ -N), ammonification, available nitrate (NO_3^- -N) and nitrous oxide (N_2O) emission were not different between fungicides and bactericides (Figure 3a,c,e,i), but nitrification was significantly higher for fungicides than for bactericides (P = 0.03, Figure 3g). Biocide types varied significantly in their effect on NH_4^+ -N, ammonification and nitrification (P = 0.0002, Figure 3b,d,h). However, NO_3^- -N and N_2O emission did not vary among biocide types (P = 0.86 and 0.24, Figure 3f,j, respectively). Many biocide types had positive effects on NH_4^+ and ammonification, but more frequently had negative effects on nitrification and N_2O emission.

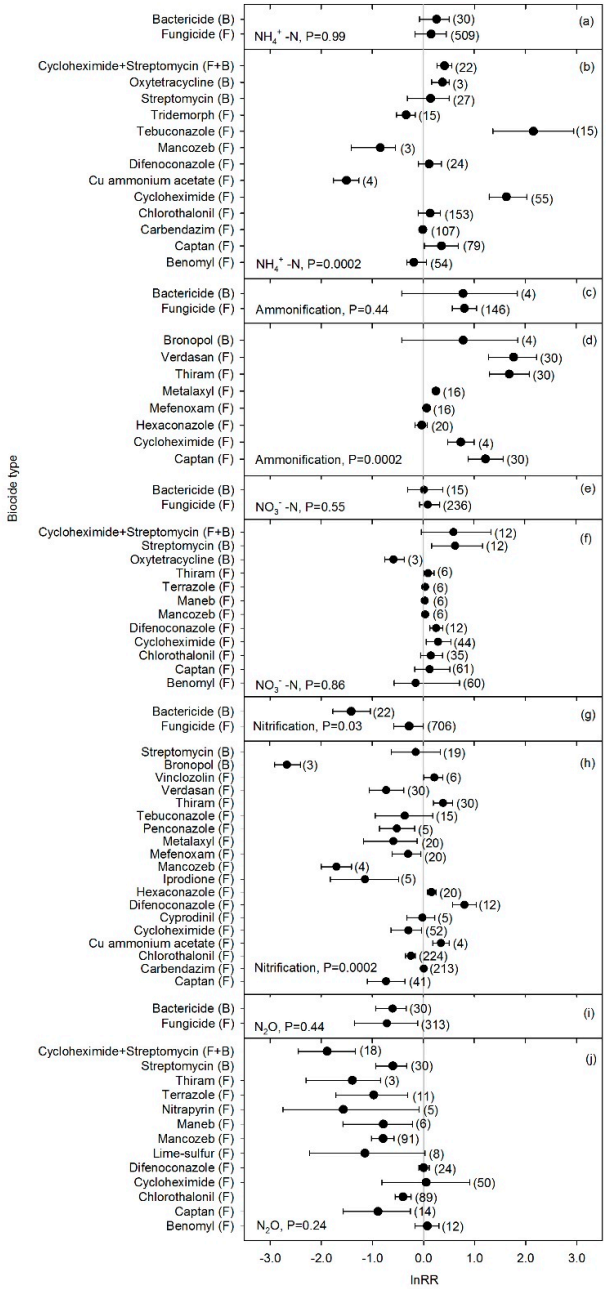

Figure 3. The mean response ratios (lnRR) of (**a**,**b**) available NH_4^+, (**c**,**d**) ammonification, (**e**,**f**) nitrate, (**g**,**h**) nitrification, and (**i**,**j**) N_2O emission to different biocide types (F: Fungicide, B: Bactericide, F+B: Fungicide + Bactericide). The error bars represent 95% bootstrapped confidence intervals (CIs). The effects of different biocides were considered significant if the 95% CI of the mean effect size did not overlap with zero. The numbers of observations for each biocide are shown next to the error bars in brackets.

We categorized the observations into five different land types: Agriculture, desert, forest, grassland, and wetland. Soil and microbial parameters for these land types varied in their responses to biocide treatments. Fungicides had negative effects on MBC for wetlands, grasslands, forests, and agricultural lands, with the most negative effect in wetlands (Figure 4a). On the other hand, bactericides had positive effects in agricultural lands (Figure 4b). Fungicides and bactericides had similar effects on soil respiration, with negative effects in wetlands and positive effects in forests (Figure 4c,d).

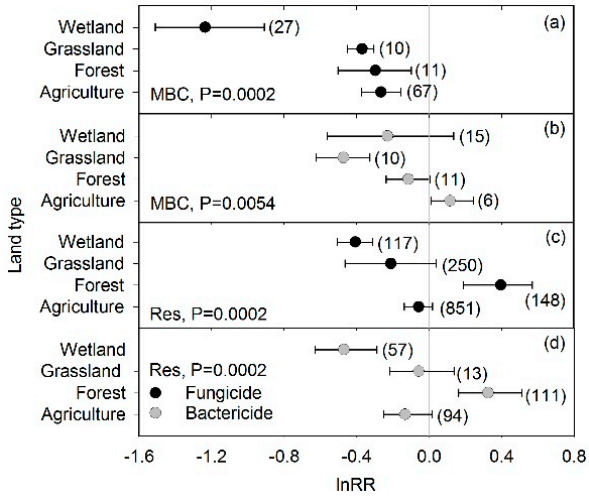

Figure 4. The mean response ratios (lnRR) of (**a**,**b**) microbial biomass C (MBC) and (**c**,**d**) soil respiration (Res) to fungicides (**a**,**c**) and bactericides (**b**,**d**) in different land types. The error bars represent 95% bootstrapped confidence intervals (CIs). The effects of different biocides were considered significant if the 95% CI of the mean effect size did not overlap with zero. The numbers of observations for each biocide are shown next to the error bars in brackets.

The effects of fungicides and bactericides on available NH_4^+, ammonification, nitrification, and N_2O emission varied significantly among land types (Figure 5a,b,e–g), but there were no land type effects on NO_3^- effect sizes (Figure 5c,d). Available NH_4^+ and ammonification were positively affected by fungicides in wetlands, grasslands, and forests, but not in agricultural lands (Figure 5a,b), while not enough data were available to examine land type effects on bactericide effect sizes. On the other hand, negative effect sizes were observed for nitrification in deserts and agricultural lands, and for N_2O emission in grasslands in response to fungicides (Figure 5e,g). The limited data for bactericides also showed negative effects on nitrification in forests, but positive effects in deserts and grasslands (Figure 5f).

The effect sizes of available nitrate (NO_3^-) and nitrification in response to bactericides were positively related to soil pH, while the effect sizes in response to fungicides did not show a significant relationship with soil pH (Figure 6).

Interestingly, effect sizes of available NO_3^- and nitrification in response to bactericides were negatively related to soil C content, with no clear relationship for the fungicide effect sizes (Figure 7). Effects sizes of soil respiration, microbial biomass C and N, and N_2O emission were not significantly related to pH or soil C content for both fungicides and bactericides. We note that the larger number of types of fungicides with observations covering a larger range in soil pH and soil C compared to the bactericides may have contributed to the lack of finding significant relationships.

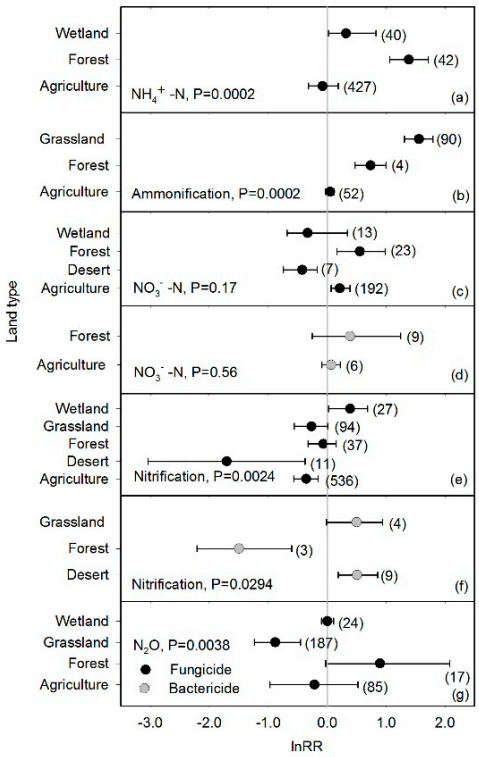

Figure 5. Meta-analysis results of the responses of (**a**) available NH_4^+, (**b**) ammonification, (**c**,**d**) nitrate, (**e**,**f**) nitrification, and (**g**) N_2O to different land types under the fungicide and bactericide treatments. The error bars represent 95% bootstrapped confidence intervals (CIs). The effects of different biocides were considered significant if the 95% CI of the mean effect size did not overlap with zero. The numbers of observations for each biocide are shown next to the error bars in brackets.

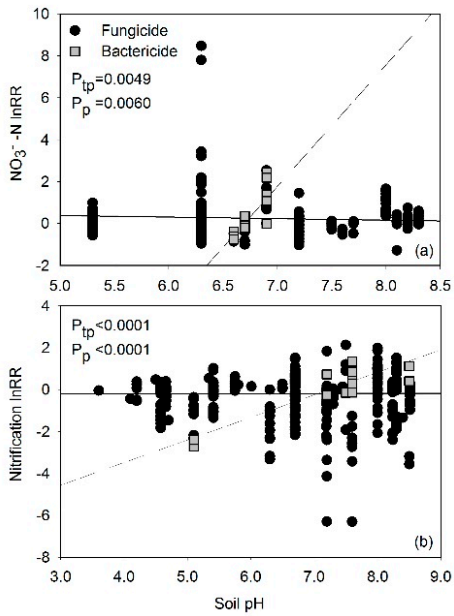

Figure 6. Relationships between soil pH and response ratios (lnRR) of (**a**) nitrate (NO_3^-) and (**b**) nitrification in response to fungicides and bactericides. Significant relationships were found between response ratios and soil pH (P_p) and interactions between biocide type and soil pH (P_{tp}).

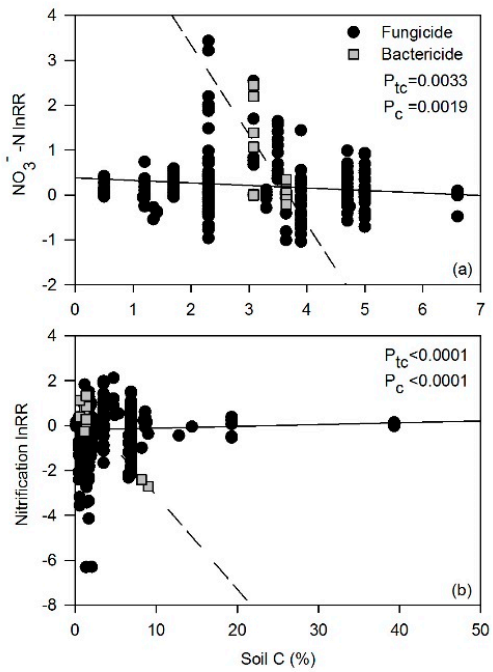

Figure 7. Relationships between soil C and response ratios (lnRR) of (**a**) nitrate (NO_3^-) and (**b**) nitrification in response to fungicides and bactericides. Significant relationships were found between response ratios and soil C (P_c) and interactions between biocide type and soil C (P_{tc}).

4. Discussion

4.1. Biocide Effects on Microbial Biomass and C-Cycle Processes

Our meta-analysis demonstrated that there were often large differences among the different biocides, but when grouped into biocide types such as fungicides and bactericides, differences were not apparent (Figure 1, Figure 2, and Figure 3). We therefore focus our discussion on different biocide type effects rather than on differences between fungicides and bactericides. Biocides had different effects on soil C and N dynamics, including microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), soil respiration, and various N-cycle processes. Most of the biocides had negative effects on MBC (Figure 1b), suggesting that these biocides were effective in limiting microbial populations. In our study, these negative impacts were observed for most of the biocides, but some were more effective than others. Tebuconazole, a broad spectrum and systemic fungicide, had the highest negative effect on MBC. Tebuconazole was also found to be persistent in soil [46]. Therefore, persistent biocides may be more effective than short-lived biocides in affecting targeted microorganisms, although long term biocide residence in soil has the potential to indirectly affect non-target soil microorganisms [47].

Furthermore, we found that most biocides had a negative effect on MBN (Figure 1d). However, an exception was benomyl (F), which showed a positive effect on MBN. One reason could be that a pulse in the fungal necromass after benomyl addition may have provided an N source to other microorganisms not affected by benomyl [34]. Interestingly, benomyl had among the highest N concentrations (19.3%) of the biocides we examined (Table S3). It is therefore also possible that benomyl may have been a source of N after its decomposition that could be used by other surviving microorganisms. Similarly, when we related MBC responses to biocide amounts applied to soil, we observed a significant positive relationship from negative effect sizes at low doses to positive effect sizes at high doses (Figure S2). This suggests that the organic biocides could be used as C sources for the surviving microorganisms, particularly at high doses, but this would also depend on the persistence (or degradability) of biocides.

Soil respiration was affected by biocide type, but it did not differ between fungicides and bactericides. As discussed above for MBC, differences in respiration effects among biocides may have occurred because some biocides are more persistent than others (e.g., References [34,47]). However, for

the majority of observations, the largest effects occurred after hours or a few days. When we related soil respiration effect sizes to incubation time, we observed the largest effects during short incubation times (Figure S3), while biocide effects on respiration rapidly declined with time. Similar results were obtained for MBC and MBN (data not shown). This decline could be due to microbial adaptation and tolerance to biocides as time elapsed [38]. However, a more likely explanation is that biocide concentrations decreased with time due to degradation, thereby reducing their impact.

4.2. Biocide Effects on N-cycle Processes

In our meta-analysis, we observed that biocides had mixed effects on N pools and transformation processes. Some fungicides caused the highest positive effect on available NH_4^+ and ammonification (Figure 3b,d), while the largest negative effect on nitrification was caused by the bactericide bronopol (Figure 3h). The reasons for these variable effects could be that (i) different soil microorganisms responsible for N transformations respond differently to various biocides, (ii) some biocide types are more persistent in soil than others, and (iii) biocide types vary in their N content and can act as an N source for surviving microorganisms. For tebuconazole (F), two of these reasons could be responsible for the highest positive effect on available soil NH_4^+: It is persistent in soil and it has a high N concentration (13.7%, Table S3). Susceptibility of nitrifying bacteria and changes in the microbial community during application of specific fungicides such as captan [48] and cycloheximide [49] could also be responsible for the positive effect on available soil NH_4^+. Moreover, most of the fungicides had a positive effect on net ammonification, possibly because application of these biocides reduced microbial NH_4^+ uptake and protein synthesis. Increased production of NH_4^+ from N mineralization from organic matter with biocide application is also possible due to the presence of a physiologically-diversified group of ammonifiers (bacteria, fungi, and some actinomycetes) that vary in their susceptibility to different biocides [21].

While most of the biocides had a negative effect on nitrification and N_2O emission processes, some biocides had positive and no effects on these processes (Figure 3h,j). Interestingly, a large number of fungicides decreased nitrification, although the traditional view is that nitrification in soil is mostly performed by autotrophic bacteria [50]. Indeed, in our meta-analysis, the bactericide bronopol had the strongest negative effect on nitrification, suggesting that nitrification was largely controlled by nitrifying bacteria. However, heterotrophic nitrification by fungi may also be important in certain soils (e.g., Reference [51]), and our results indicate that many fungicides can significantly suppress nitrification. Previous studies have also shown that bacteria were a main agent for denitrification and N_2O emission [52,53]. However, our meta-analysis revealed that many fungicides negatively affected N_2O emission, and it has become increasingly clear that fungi from various phyla are known to be capable of denitrification and N_2O production [54,55]. However, a significant response (positive or negative) on N_2O emission was absent for the fungicides benomyl, cycloheximide and difenoconazole. Possible reasons for this lack of effect are (i) variation in biocide amounts and incubation duration, and (ii) that fungicide applications provided significant C and N sources for denitrifying microorganisms thereby offsetting any negative effects on N_2O emission. Several studies showed that low amounts of these biocides had non-significant effects on denitrification [49,56]. It was suggested that difenoconazole (N = 10.4%, C/N = 5) acted as an alternative C source for denitrifying microorganisms and was responsible for denitrification rates [56]. While the application of a low amount of cycloheximide reduced N_2O production by 69–99% [57], long-term presence of this biocide in soil was responsible for a positive effect on N_2O emission due to the decomposition of dead microbes or biocides for other microorganisms [58].

4.3. Fungicide and Bactericide Effects in Relation to Land Type

Although there were in general no differences between fungicides and bactericides on C and N cycling parameters, the extent of the influence of fungicides and bactericides on soil and microbial parameters depended on major land types. We found that MBC, respiration, and four N-cycling

parameters (available NH_4^+, ammonification, nitrification, and N_2O) varied among five different land types (agriculture, desert, forest, grassland, and wetland) in response to fungicide and bactericide treatments (Figures 4 and 5). These variable responses could be due to differences in microbial community structure and soil characteristics such as pH, C content (see below), and nutrient content among land types.

For instance, when the abundance of fungal communities is relatively low, then it can be expected that the effect of fungicides on C and N cycling would also be low. This could explain the relatively low and sometimes lack of effect of fungicides on MBC, respiration, available NH_4^+, ammonification, nitrification, and N_2O emission in agricultural lands. Agricultural lands often have relatively low fungal abundances due to factors such as disturbance through tillage, use of non-mycorrhizal crops, and high levels of soil nutrients that suppress fungal growth [59]. If, as discussed above, the fungicides and bactericides themselves are a source of N, then the application of these biocides would have relatively small effects on the N cycle in systems where N is already abundant and transformed quickly as in most agricultural soils.

Interestingly, fungicides and bactericides showed some of the strongest negative effects on MBC (fungicides only) and respiration in wetlands (Figure 4). Because wetlands tend to have relatively high soil C and MBC contents [60], they therefore may be more sensitive to biocides compared to the other land types. High inputs of dissolved organic matter from different sources in this land type can promote large microbial growth and activity, both fungi and bacteria [60,61]. Therefore, reductions in respiration caused by biocides may be particularly strong in this land type.

In contrast, the application of fungicides and bactericides had positive effects on respiration, available NH_4^+, ammonification, available NO_3^- (fungicides only), and N_2O emission in forests. High microbial diversity and availability of a broad range of substrate types in forest soils [62] compared to other land types could be responsible for these effects. Changes in the supply of specific substrates could therefore enhance the activity of microbial groups specialized in these substrates. The death of specific microbial groups through application of fungicides and bactericides may then have provided a substrate for specific non-targeted microorganisms. This could also be a plausible reason for the large increase in ammonification in response to fungicide application in grasslands (Figure 5b). In contrast, fungicides had a negative effect on N_2O emission in grasslands. As discussed above, fungi can be an important contributor to N_2O emission [55], and our results here suggest that fungi in grasslands may be particularly important for N_2O production.

In desert soil, fungicides showed the highest negative effect, while bactericides showed the highest positive effect on nitrification (Figure 5e,f). Fungi may be able to survive in less fertile and low moisture in desert soils, whereas bacterial growth and activity are often restricted in extreme dryness [63]. Others have suggested that N transformations in desert soil are mainly dominated by fungi [64] because of their capacity to metabolize at low water potentials [65]. Unfortunately, there was limited or no information about fungicide and bactericide effects on other C and N cycling parameters.

4.4. Fungicide and Bactericide Effects in Relation to Soil pH and C

We examined whether fungicide and bactericide effects on C and N cycling parameters depended on soil pH and C content. Fungi tend to be more dominant than bacteria in acidic soils [66], while bacterial activity and diversity tend to increase with soil pH [67]. It can therefore be argued that fungicides should cause a greater negative effect in low pH soils and bactericides in high pH soils if the sole effect of biocides is to suppress the activity of the target organisms, thereby suppressing the activity of the whole microbial community. Instead, we found that fungicide effects were independent of soil pH, but bactericide effects on available NO_3^- and nitrification were positively related to soil pH (Figure 6). Although the number of observations for bactericides is far smaller than for fungicides, the positive relationship with soil pH is intriguing. Negative effects of bactericides on available NO_3^- and nitrification at low soil pH suggests that under these conditions bactericides were effective in reducing the activity of nitrifying bacteria. On the other hand, positive effects of bactericides on available NO_3^-

and nitrification at high soil pH could suggest that bactericides were effective in killing a large number of bacteria, thereby providing a significant N source for the surviving nitrifying microbial community. It is also possible that the bactericides themselves provided an N source for nitrification (e.g., the bactericide streptomycin had high N content, Table S3), but it is unclear why this would only enhance available N and nitrification at high soil pH. Further research is warranted to test the sensitivity of biocides on C and N cycling with soil pH.

Similarly, we observed that the effects of bactericides on available NO_3^- and nitrification were more sensitive to variations in soil C compared to the effects of fungicides (Figure 7). Again, we caution that the number of observations for bactericides was much smaller than for fungicides. In this case, bactericides had positive effects on available NO_3^- and nitrification when soil C was low, but effects became negative at higher soil C. Possibly, low C soils support low available NO_3^- and nitrification rates, and under those conditions, the addition of bactericides could provide a substantial source of N for nitrification. On the other hand, high C soils may support large bacterial communities so that application of bactericides can significantly reduce their nitrifying activity. It is unclear why we did not find these patterns for fungicides, but we note the large scatter for fungicides (also in relation to soil pH), possibly because the data are derived from a large group of different fungicide types, and as discussed above, these types can vary significantly in their effect on C and N cycling.

Our meta-analysis highlighted several limitations. First, there was a limited number of studies examining bactericide effects on C and N cycling parameters with the majority of studies related to fungicide effects, which hampered our analyses of contrasting the effects of fungicides vs. bactericides, and relating bactericide effects to soil pH and C content. Second, there was an unequal number of observations for specific biocide types. For instance, fungicides such as captan and cycloheximide and bactericides such as bronopol and streptomycin had higher numbers of observations than other biocides. Although this may not have affected our comparison of effects among biocide types since we controlled for the observation number in our meta-analysis, it may have affected our comparison of effects among different land types if certain biocide types were used more often in specific land types than others. Unfortunately, because of the low number of observations for specific biocide types in specific land types, we were unable to tease apart biocide type from land type effects. A third limitation of our study is that the number of observations varied among major land types. Of the five land types examined in our study, most of the studies were conducted in agricultural and forest land types. For some land types, observations were smaller than 10 or completely lacking (e.g., desert land type), causing us to use caution with some of the implications of our results.

5. Conclusions

Inhibition techniques with fungicides and bactericides have been used as a simple and cheap way to understand the responses of fungi and bacteria to C- and N-cycling processes. However, for this purpose, it is important to select suitable fungicides and bactericides that have a significant effect on target microorganisms and no- or limited impact on non-target microorganisms. Previous studies have shown that fungicides and bactericides are not always effective in inhibiting the activity of fungi and bacteria, respectively, but could directly reduce the activity of non-targeted microorganisms as well (e.g., References [35,49]). From our meta-analysis, we found large variation among different fungicides and bactericides on C and N cycling parameters. On several occasions, fungicides and bactericides caused positive effects on C and N cycling (respiration, available NH_4^+, and ammonification), despite their intent to reduce microbial C and N cycling by fungi and bacteria, respectively. We offer several explanations for the wide variation in the effects caused by biocides. First, biocides may differ in their effectiveness in killing target organisms, which may also depend on the rate of and time since biocide application. Second, biocides targeting specific microbial groups may shift competition for C and nutrient resources among microbes, thereby stimulating the activity of specific non-targeted microbes depending on the biocide type. Third, microbes killed by biocides may become C and N sources of surviving microbes, thereby affecting C and N cycling depending on the biocide type. Fourth, biocides

themselves may become sources of C and N for surviving microbes, resulting in different effects among biocide types because of their differences in C and N content and in their decomposability. Finally, biocides may cause variable effects on C and N cycling because their effects depend on the land type and soil characteristics such as pH and C content.

We observed large differences in the effects of fungicides and bactericides on C and N cycling parameters among land types. For instance, wetlands showed some of the largest decreases in MBC and respiration in response to fungicides and bactericides, possibly because this land type can support relatively large amounts of microbial biomass. In contrast, C and N cycling parameters in agricultural soils were, in general, less responsive to fungicides, possibly because these soils tend to support smaller amounts of fungal biomass, and agricultural soils usually have relatively high rates of N transformation that are mediated by bacteria rather than fungi. We further observed that the effects of bactericides on available NO_3^- and nitrification were more sensitive to soil pH and C content than the effects of fungicides. These effects shifted from negative to positive along the soil pH gradient, and from positive to negative along the soil C gradient, suggesting that different mechanisms operated along these gradients.

We believe our meta-analysis provides insightful information about the use of specific fungicides and bactericides in understanding their effects on C and N cycling in soils. While fungicide and bactericide applications often resulted in significant reductions in microbial biomass C and N, they sometimes caused surprising effects on respiration and N transformation rates. We therefore caution the use of fungicides and bactericides with the intention to understand the role of fungal and bacterial activity, respectively, on C and N cycling, and suggest that their effects on microbial community shifts and C and N availability need to be considered for a better understanding of the role of fungi and bacteria for C and N cycling in soils.

Supplementary Materials: The following are available online at http://www.mdpi.com/2571-8789/3/2/23/s1, Spreadsheet S1: Supplementary data, Figure S1: Number of publications with time used in our meta-analysis, Figure S2: The response ratio of MBC in relation to biocide amount, Figure S3: The response ratio of soil respiration in relation to incubation time, Table S1: Selected keywords for this meta-analysis, Table S2: Country-wise application of different biocides, Table S3: Chemical structure of studied biocides, References S1: References used in meta-analysis.

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References

1. Ågren, G.I. Climate change: Microbial mitigation. *Nat. Geosci.* **2010**, *3*, 303–304. [CrossRef]
2. Schimel, J.P.; Bennett, J. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* **2004**, *85*, 591–602. [CrossRef]
3. Prosser, J.I.; Bohannan, B.J.M.; Curtis, T.P.; Ellis, R.J.; Firestone, M.K.; Freckleton, R.P.; Green, J.L.; Green, L.E.; Killham, K.; Lennon, J.J. The role of ecological theory in microbial ecology. *Nat. Rev. Microbiol.* **2007**, *5*, 384. [CrossRef] [PubMed]
4. Bååth, E.; Anderson, T.-H. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol. Biochem.* **2003**, *35*, 955–963. [CrossRef]
5. Fierer, N.; Strickland, M.S.; Liptzin, D.; Bradford, M.A.; Cleveland, C.C. Global patterns in belowground communities. *Ecol. Lett.* **2009**, *12*, 1238–1249. [CrossRef] [PubMed]
6. Bardgett, R.D.; Freeman, C.; Ostle, N.J. Microbial contributions to climate change through carbon cycle feedbacks. *ISME J.* **2008**, *2*, 805–814. [CrossRef] [PubMed]

7. Bailey, V.L.; Smith, J.L.; Bolton, H., Jr. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biol. Biochem.* **2002**, *34*, 997–1007. [CrossRef]
8. Milne, R.M.; Haynes, R.J. Soil organic matter, microbial properties, and aggregate stability under annual and perennial pastures. *Biol. Fertil. Soils* **2004**, *39*, 172–178. [CrossRef]
9. Liang, C.; Balser, T.C. Microbial production of recalcitrant organic matter in global soils: Implications for productivity and climate policy. *Nat. Rev. Microbiol.* **2011**, *9*, 75. [CrossRef]
10. Schimel, J.; Schaeffer, S.M. Microbial control over carbon cycling in soil. *Front. Microbiol.* **2012**, *3*, 348. [CrossRef]
11. Schurig, C.; Smittenberg, R.H.; Berger, J.; Kraft, F.; Woche, S.K.; Goebel, M.-O.; Heipieper, H.J.; Miltner, A.; Kaestner, M. Microbial cell-envelope fragments and the formation of soil organic matter: A case study from a glacier forefield. *Biogeochemistry* **2013**, *113*, 595–612. [CrossRef]
12. De Gryze, S.; Six, J.; Brits, C.; Merckx, R. A quantification of short-term macroaggregate dynamics: Influences of wheat residue input and texture. *Soil Biol. Biochem.* **2005**, *37*, 55–66. [CrossRef]
13. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 555–569. [CrossRef]
14. Zechmeister-Boltenstern, S.; Keiblinger, K.M.; Mooshammer, M.; Peñuelas, J.; Richter, A.; Sardans, J.; Wanek, W. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. *Ecol. Monogr.* **2015**, *85*, 133–155. [CrossRef]
15. Zhou, Z.; Wang, C.; Zheng, M.; Jiang, L.; Luo, Y. Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biol. Biochem.* **2017**, *115*, 433–441. [CrossRef]
16. Lladó, S.; López-Mondéjar, R.; Baldrian, P. Forest soil bacteria: Diversity, involvement in ecosystem processes, and response to global change. *Microbiol. Mol. Biol. Rev.* **2017**, *81*, e00063-16. [CrossRef] [PubMed]
17. Strickland, M.S.; Rousk, J. Considering fungal: Bacterial dominance in soils–methods, controls, and ecosystem implications. *Soil Biol. Biochem.* **2010**, *42*, 1385–1395. [CrossRef]
18. Rousk, J.; Bååth, E. Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiol. Ecol.* **2011**, *78*, 17–30. [CrossRef] [PubMed]
19. Högberg, M.N.; Högberg, P.; Myrold, D.D. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* **2007**, *150*, 590–601. [CrossRef] [PubMed]
20. Firestone, M.K.; Davidson, E.A. Microbiological basis of NO and N2O production and consumption in soils. In *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*; Andreae, M.O., Schimel, D.S., Eds.; John Wiley and Sons: New York, NY, USA, 1989; Volume 47, pp. 7–21.
21. Boyle, S.A.; Yarwood, R.R.; Bottomley, P.J.; Myrold, D.D. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. *Soil Biol. Biochem.* **2008**, *40*, 443–451. [CrossRef]
22. Hart, S.C.; Binkley, D.; Perry, D.A. Influence of red alder on soil nitrogen transformations in two conifer forests of contrasting productivity. *Soil Biol. Biochem.* **1997**, *29*, 1111–1123. [CrossRef]
23. Merrick, M.J.; Edwards, R.A. Nitrogen control in bacteria. *Microbiol. Rev.* **1995**, *59*, 604–622.
24. Marzluf, G.A. Genetic regulation of nitrogen metabolism in the fungi. *Microbiol Mol. Biol. Rev.* **1997**, *61*, 17–32.
25. Chen, J.; Stark, J.M. Plant species effects and carbon and nitrogen cycling in a sagebrush–crested wheatgrass soil. *Soil Biol. Biochem.* **2000**, *32*, 47–57. [CrossRef]
26. Ponge, J.F. Humus forms in terrestrial ecosystems: A framework to biodiversity. *Soil Biol. Biochem.* **2003**, *35*, 935–945. [CrossRef]
27. De Vries, F.T.; Van Groenigen, J.W.; Hoffland, E.; Bloem, J. Nitrogen losses from two grassland soils with different fungal biomass. *Soil Biol. Biochem.* **2011**, *43*, 997–1005. [CrossRef]
28. Pennanen, T.; Liski, J.; Bååth, E.; Kitunen, V.; Uotila, J.; Westman, C.J.; Fritze, H. Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. *Microbiol. Ecol.* **1999**, *38*, 168–179. [CrossRef]
29. Leckie, S.E.; Prescott, C.E.; Grayston, S.J.; Neufeld, J.D.; Mohn, W.W. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. *Microbiol. Ecol.* **2004**, *48*, 29–40. [CrossRef]
30. Rousk, J.; Demoling, L.A.; Bahr, A.; Bååth, E. Examining the fungal and bacterial niche overlap using selective inhibitors in soil. *FEMS Microbiol. Ecol.* **2008**, *63*, 350–358. [CrossRef]

31. McManus, P.S.; Stockwell, V.O.; Sundin, G.W.; Jones, A.L. Antibiotic use in plant agriculture. *Annu. Rev. Phytopathol.* **2002**, *40*, 443–465. [CrossRef]
32. Piotrowska-Seget, Z.; Engel, R.; Nowak, E.; Kozdrój, J. Successive soil treatment with captan or oxytetracycline affects non-target microorganisms. *World J. Microbiol. Biotechnol.* **2008**, *24*, 2843. [CrossRef]
33. Milenkovski, S.; Bååth, E.; Lindgren, P.E.; Berglund, O. Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. *Ecotoxicology* **2010**, *19*, 285–294. [CrossRef]
34. Chen, S.-K.; Edwards, C.A.; Subler, S. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biol. Biochem.* **2001**, *33*, 1971–1980. [CrossRef]
35. Bailey, V.L.; Smith, J.L.; Bolton, H. Novel antibiotics as inhibitors for the selective respiratory inhibition method of measuring fungal:bacterial ratios in soil. *Biol. Fertil. Soils* **2003**, *38*, 154–160. [CrossRef]
36. Rousk, J.; Demoling, L.A.; Bååth, E. Contrasting short-term antibiotic effects on respiration and bacterial growth compromises the validity of the selective respiratory inhibition technique to distinguish fungi and bacteria. *Microb. Ecol.* **2009**, *58*, 75–85. [CrossRef]
37. Wang, C.; Wang, F.; Zhang, Q.; Liang, W. Individual and combined effects of tebuconazole and carbendazim on soil microbial activity. *Eur. J. Soil Biol.* **2016**, *72*, 6–13. [CrossRef]
38. Ju, C.; Xu, J.; Wu, X.; Dong, F.; Liu, X.; Tian, C.; Zheng, Y. Effects of hexaconazole application on soil microbes community and nitrogen transformations in paddy soils. *Sci. Total Environ.* **2017**, *609*, 655–663. [CrossRef]
39. Kalia, A.; Gosal, S.K. Effect of pesticide application on soil microorganisms. *Arch. Agron. Soil Sci.* **2011**, *57*, 569–596. [CrossRef]
40. Hedges, L.V.; Gurevitch, J.; Curtis, P.S. The meta-analysis of response ratios in experimental ecology. *Ecology* **1999**, *80*, 1150–1156. [CrossRef]
41. Rosenberg, M.S.; Adams, D.C.; Gurevitch, J. *MetaWin: Statistical Software for Meta-Analysis*; Sinauer Associates: Sunderland, MA, USA, 2000.
42. Dijkstra, F.A.; Adams, M.A. Fire eases imbalances of nitrogen and phosphorus in woody plants. *Ecosystems* **2015**, *18*, 769–779. [CrossRef]
43. Hedges, L.V.; Olkin, I. *Statistical Methods for Meta-Analysis*; Academic Press, Inc.: Orlando, FL, USA, 1985.
44. Cardinale, B.J.; Srivastava, D.S.; Duffy, J.E.; Wright, J.P.; Downing, A.L.; Sankaran, M.; Jouseau, C. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* **2006**, *443*, 989–992. [CrossRef]
45. Van Groenigen, K.J.; Osenberg, C.W.; Hungate, B.A. Increased soil emissions of potent greenhouse gases under increased atmospheric CO_2. *Nature* **2011**, *475*, 214–216. [CrossRef]
46. Strickland, T.C.; Potter, T.L.; Joo, H. Tebuconazole dissipation and metabolism in Tifton loamy sand during laboratory incubation. *Pest Manag. Sci.* **2004**, *60*, 703–709. [CrossRef]
47. Muñoz-Leoz, B.; Ruiz-Romera, E.; Antigüedad, I.; Garbisu, C. Tebuconazole application decreases soil microbial biomass and activity. *Soil Biol. Biochem.* **2011**, *43*, 2176–2183. [CrossRef]
48. Chen, S.-K.; Edwards, C.A.; Subler, S. A microcosm approach for evaluating the effects of the fungicides benomyl and captan on soil ecological processes and plant growth. *Appl. Soil Ecol.* **2001**, *18*, 69–82. [CrossRef]
49. Castaldi, S.; Smith, K.A. Effect of cycloheximide on N_2O and NO_3^- production in a forest and an agricultural soil. *Biol. Fertil. Soils* **1998**, *27*, 27–34. [CrossRef]
50. Isobe, K.; Koba, K.; Otsuka, S.; Senoo, K. Nitrification and nitrifying microbial communities in forest soils. *J. For. Res.* **2011**, *16*, 351–362. [CrossRef]
51. Zhu, T.; Meng, T.; Zhang, J.; Zhong, W.; Müller, C.; Cai, Z. Fungi-dominant heterotrophic nitrification in a subtropical forest soil of China. *J. Soils Sediments* **2015**, *15*, 705–709. [CrossRef]
52. Kampschreur, M.J.; Temmink, H.; Kleerebezemm, R.; Jetten, M.S.M.; van Loosdrecht, M.C.M. Nitrous oxide emission during wastewater treatment. *Water Res.* **2009**, *43*, 4093–4103. [CrossRef]
53. Shen, J.P.; Xu, Z.; He, J.Z. Frontiers in the microbial processes of ammonia oxidation in soils and sediments. *J. Soils Sediments* **2014**, *14*, 1023–1029. [CrossRef]
54. Maeda, K.; Spor, A.; Edel-Hermann, V.; Heraud, C.; Breuil, M.-C.; Bizouard, F.; Toyoda, S.; Yoshida, N.; Steinberg, C.; Philippot, L. N_2O production, a widespread trait in fungi. *Sci. Rep.* **2015**, *5*, 19697–19704. [CrossRef] [PubMed]
55. Marusenko, Y.; Huber, D.P.; Hall, S.J. Fungi mediate nitrous oxide production but not ammonia oxidation in aridland soils of the southwestern US. *Soil Biol. Biochem.* **2013**, *63*, 24–36. [CrossRef]

56. Muñoz-Leoz, B.; Garbisu, C.; Charcosset, J.-Y.; Sánchez-Pérez, J.M.; Antigüedad, I.; Ruiz-Romera, E. Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil. *Sci. Total Environ.* **2013**, *449*, 345–354. [CrossRef] [PubMed]
57. Ananyeva, N.D.; Ivashchenko, K.V.; Stolnikova, E.V.; Stepanov, A.L.; Kudeyarov, V.N. Specific features of determination of the net production of nitrous oxide by soils. *Eurasian Soil Sci.* **2015**, *48*, 608–619. [CrossRef]
58. Ma, S.; Shan, J.; Yan, X. N_2O emissions dominated by fungi in an intensively managed vegetable field converted from wheat–rice rotation. *Appl. Soil Ecol.* **2017**, *116*, 23–29. [CrossRef]
59. Johansson, J.F.; Paul, L.R.; Finlay, R.D. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.* **2004**, *48*, 1–13. [CrossRef] [PubMed]
60. Kayranli, B.; Scholz, M.; Mustafa, A.; Hedmark, Å. Carbon storage and fluxes within freshwater wetlands: A critical review. *Wetlands* **2010**, *30*, 111–124. [CrossRef]
61. Reddy, K.R.; DeLaune, R.D. *Biogeochemistry of Wetlands: Science and Applications*, 1st ed.; CRC press, Taylor and Francis Group: Boca Raton, FL, USA, 2008; p. 800.
62. Baldrian, P. Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiol. Rev.* **2017**, *41*, 109–130. [CrossRef] [PubMed]
63. Rao, S.; Chan, Y.; Bugler-Lacap, D.C.; Bhatnagar, A.; Bhatnagar, M.; Pointing, S.B. Microbial diversity in soil, sand dune and rock substrates of the Thar Monsoon Desert, India. *Indian J. Microbiol.* **2016**, *56*, 35–45. [CrossRef]
64. Crenshaw, C.L.; Lauber, C.; Sinsabaugh, R.L.; Stavely, L.K. Fungal control of nitrous oxide production in semiarid grassland. *Biogeochemistry* **2008**, *87*, 17–27. [CrossRef]
65. McLain, J.E.T.; Martens, D.A. N_2O production by heterotrophic N transformations in a semiarid soil. *Appl. Soil Ecol.* **2006**, *32*, 253–263. [CrossRef]
66. Rousk, J.; Brookes, P.C.; Bååth, E. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* **2009**, *75*, 1589–1596. [CrossRef] [PubMed]
67. Rousk, J.; Bååth, E.; Brookes, P.C.; Lauber, C.L.; Lozupone, C.; Caporaso, J.G.; Knight, R.; Fierer, N. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **2010**, *4*, 1340–1351. [CrossRef] [PubMed]