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Carbon Addition Modified the Response of Heterotrophic Respiration to Soil Sieving in Ectomycorrhizal-Dominated Forests

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Abstract: Soil heterotrophic respiration (R_h) is an important pathway of carbon (C) dioxide release from terrestrial soils to the atmosphere. It is often measured using sieved soil in a laboratory, but the uncertainty of how it is influenced by soil sieving persists, which limits the accuracy of predicting soil organic C dynamics in C models. To address how soil sieving during laboratory incubation affects R_h and its response to increased carbon availability, we investigated R_h in sieved and intact soil cores and its response to ^{13}C-glucose addition. This was conducted through a 27-day laboratory incubation in four forests, including two ectomycorrhizal-dominated (ECM) forests and two arbuscular mycorrhizal-dominated forests. The significant influence of soil sieving on R_h in all forests was not observed during incubation when glucose was not added. After adding glucose, the R_h in the sieved soils on the 5th day of incubation was averaged 27.2% lower than that in intact soils in ECM forests. On the 27th day it was 22.1% lower in the *Pinus massoniana* forest, but 78.0% higher in the *Castanea mollissima* forest. Strong relationships were detected between R_h in sieved and intact soils (r^2 = 0.888), and in soils both with and without the addition of glucose (r^2 = 0.827). The measured soil variables explained 74.7% and 49.7% of the variation in R_h on the 5th and 27th day of incubation, and the role of soil nutrients and microbial PLFA groups in regulating R_h varied temporally. Our findings suggest that plant mycorrhizal types influenced the role of increased C availability to microbes in regulating the response of R_h to sieving in forest ecosystems.

Keywords: soil heterotrophic respiration; soil sieving; intact soil core; carbon sequestration; microbial community; plant functional type

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1. Introduction

Globally, soils contain 1500 Pg of organic carbon (C) in the 1 m depth, which is approximately 60% of C stocks in terrestrial ecosystems [1]. Thus, any small change in soil C flux will have a great influence on atmospheric carbon dioxide (CO_2) concentration and the feedback to global climate change [2]. Soil respiration, the second largest C flux between terrestrial ecosystem and atmosphere, has been estimated to be 60–100 Pg C yr^{-1}, more than all anthropogenic sources combined [3]. More than half of soil respiration is from heterotrophic respiration (R_h) produced by the microbial decay of soil organic C (SOC) [3,4]. Therefore, measuring soil R_h is important for quantifying the CO_2 flux from soils to the atmosphere and enhancing the accuracy of the SOC dynamics predicted by C models [5].

As a common practice, soil sieving is usually conducted prior to laboratory incubation when researching soil C and N cycles, including soil R_h [6–9]. However, sieving disrupts the soil's physical structure such as soil aggregates and porosity [10]. This increases the exposure of SOC physically protected within aggregates and the oxygen availability to microbes [11–13], thereby affecting soil R_h. Therefore, R_h derived from sieved soils may

not be representative of true values occurring in field conditions. In comparison to sieved soils, using intact field-moist soil cores for determining soil R_h has been suggested to better reflect the actual field conditions [4]. However, there is still no consistent conclusion on the influence of soil R_h sieving relative to intact soil cores. This indicated that sieved soils had higher [12,14,15], similar to, or lower soil R_h than intact soils [11,16–18], and most of them were conducted in cropland and grassland. This indicated that predicting the effects of sieving on soil R_h may be very difficult. Thus, the influence of sieving on soil R_h needs to be more clearly defined in forest ecosystems.

Given that soil R_h is mostly the activity of soil microbes and is strongly limited by C availability to soil microbes [19–21], the conflicting responses of soil R_h to sieving in the above-mentioned studies may be explained by differences in C availability. Carbon addition has been widely used to increase soil C availability, and has significantly changed the microbial activity (e.g., respiration and enzyme activity) and community composition [8,22,23]. Although the effects of C or substrate addition on soil R_h or SOC decomposition using sieved soils were widely investigated [8,13,24,25], their changes caused by C addition in sieved soil cores and intact soil cores were less explored. Stenger et al. [18] found that added glucose-C decomposition in intact soils was similar to that in sieved soils, but they did not investigate how the interaction of sieving and glucose addition affected R_h. Thus, it is less clear how increased C availability to soil microbes through glucose addition in soils mediates the response of R_h to sieving through directly and/or indirectly changing soil microbial activity and community composition.

Plant functional types (e.g., mycorrhizal type) may affect soil C availability to microbes because litters from arbuscular mycorrhizal-associated (AM) trees have a lower C:N and faster decomposition rate than ectomycorrhizal-dominated(ECM) trees [26,27]. Thus, we speculated that the effects of C addition on the responses of soil R_h to sieving would differ in ECM and AM forests. In this study, in order to explore how increasing C availability influenced the responses of R_h to soil sieving in forest ecosystems, we collected soil cores from two ECM forests and two AM forests in subtropical China. We used the laboratory incubation method to measure R_h in both sieved and intact soil cores, and then assessed the influence of soil sieving, glucose addition and their interaction on R_h. We further measured soil nutrients and microbial properties based on phospholipid acids (PLFAs) to reveal the underlying mechanisms of the influence of soil sieving and glucose addition on soil R_h. Given that sieving disrupts the soil's physical structure and increases substrate accessibility and oxygen availability to microbes [11,13], we hypothesized that sieving would stimulate soil R_h, but that the stimulatory degree would be different in ECM and AM forests. Glucose, a readily available substrate, may be preferentially used by microbes relative to the native SOC [28], so we hypothesized that increasing C availability by adding glucose would decrease the influence of sieving on soil R_h.

2. Materials and Methods

2.1. Site Description and Soil Collection

This study was conducted at the Huitong National Research Station of Forest Ecosystem (26°40′ N, 109°26′ E) in southern China. In this region, the altitude ranges from 300–1000 m. Soils that had developed from grayish-green slate parent materials are classified as Ultisol according to the second edition of the U.S. Soil Taxonomy [8]. The mean annual temperature was 16.5 °C, and the mean annual rainfall was 1200 mm over the past 20 years. The mean minimum and maximum temperature occurs in January and July, respectively. The native forests are subtropical, evergreen broadleaved forests with the dominant understory vegetation species being *Rubus rosifolius*, *Pteridium aquilinum*, *Maesa japonica*, *Parathelypteris chinensis*, and *Microlepia marginata*, but most have been destroyed and replaced by other forests.

We collected soil cores from two ECM pure forests (i.e., *Castanea mollissima* and *Pinus massoniana*) and two AM pure forests (i.e., *Schima superba* and *Cunninghamia lanceolata*). For each forest type, we selected three forest stands with about 0.3 ha for each forest stand as

3 replications, and in each forest stand we established 3 plots with 10 m × 10 m. In each plot, we sampled 9 intact soil cores (with a 5 cm inner diameter and a 10 cm depth) using PVC cylinders, and immediately took them into the laboratory. After collection, 5 of the 9 soil cores from the plot in each forest were sieved through a 2 mm screen and mixed completely. Among the sieved soils, 4 of them were refilled into PVC cylinders to maintain their original bulk densities (all materials were also repacked). These were referred as sieved cores, and the remaining one was used for measuring soil chemical properties and water content. The remaining 4 of 9 intact soil cores were referred as intact cores. The base of each cylinder was sealed with plastic film to prevent any leaching losses. Both the sieved cores and intact cores were pre-incubated for 7 days at 25 °C to minimize the "pulse effect" of sieving on CO_2 release.

2.2. Soil Chemical and Microbial Analysis

Air-dried soils were ground to sieve through a 0.25 mm mesh, and then SOC and total N concentrations were determined using a C/N analyzer. Fresh soil ammonium (NH_4^+-N) and nitrate nitrogen (NO_3-N) concentrations were extracted using 2 mol L^{-1} KCl solution and determined by colorimetry, and their sum was a mineral N. Soil available phosphorus (P) was colorimetrically determined using the molybdate blue method after soil was extracted with a 1 mol L^{-1} NH_4F solution. Soil pH was determined with a pH meter from soil slurry with a 1:2.5 ratio of soil and deionized water (weight:volume). Soil bulk density was measured using soil core that was dried in an oven to a constant weight (105 °C). The standard laboratory analysis methods were seen in Lu [29]. Some of the soil key properties are presented in Table 1. Soil microbial biomass and community composition was assessed using phospholipid acids according to the method described by White and Ringelberg [30]. Methyl nonadecanoate (19:0) was added as the internal standard for quantifying the PLFAs. The assignment of PLFA to different main microbial groups was according to the method of Joergensen [31] and is listed in Table S1.

Table 1. The soil physico-chemical properties in four forests before incubation.

Forest	SOC (g kg $^{-1}$)	Total N (g kg^{-1})	C:N	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	AP (mg kg^{-1})	pH	BD (g cm^3)
CM	12.9 c	1.33 b	9.7 c	29.5 c	7.1 c	9.5 b	4.28 b	1.28 a
PM	36.5 a	2.47 a	14.5 b	34.7 bc	23.5 a	10.3 b	4.07 b	1.02 c
SS	34.7 a	2.62 a	23.3 a	55.4 a	6.8 c	4.61 c	4.27 b	1.12 bc
CL	20.9 b	1.62 b	12.9 b	38.6 b	17.0 b	39.5 a	4.70 a	1.19 b

SOC, AP and BD represent soil organic carbon, available P and bulk density, respectively. CM, PM, SS and CL denote *Castanea mollissima*, *Pinus massoniana*, *Schima superba* and *Cunninghamia lanceolata* forests, respectively. Letters followed data in the same column denote significant difference among forests.

2.3. Soil Incubation

After pre-incubation of both the sieved and intact cores, the uniformly labelled ^{13}C-glucose as a water solution (2 mL) was added into a half of the sieved and intact cores using asyringe to increase C availability. An equal amount of deionized water was added into the remaining soil cores. The amount of added glucose ($\delta^{13}C$ = 299.8‰) was equal to the 2% of the SOC content. All soil cores were placed into 1000 mL Mason jars with airtight lids, with two small pores to avoid too high a CO_2 concentration during incubation. They were incubated for 27 days at 16.0 °C, which reflected the average temperature of topsoil (at a 5 cm depth). During incubation, the soil water content was maintained at 60% of the water-holding capacity through adding deionized water at intervals. The gas in the Mason jars was collected on the 1st, 3rd, 5th, 10th, 17th and 27th day of incubation. The amount of respired CO_2 and its ^{13}C value was analyzed using a stable isotope-ratio mass spectrometer. Before collection, the gas in the Mason jars was replaced by air without CO_2 and then sealed for 8–12 h. On the 5th day of incubation, half of the sieved and intact soil

cores with and without glucose addition were harvested in order to determine the soil mineral N, available P and PLFAs.

2.4. Data Calculation and Statistic Analysis

To calculate the amount of CO_2 derived from soil R_h (i.e., SOC decomposition) in soils with glucose addition, we used the equation [8]:

$$CRh = CT\ (\delta G - \delta T)/(\delta G - \delta S)$$

In the equation, C_T and C_{Rh} are the total amount of CO_2 and the amount of CO_2 derived from soil R_h during the considered time interval, respectively. δ_T, δ_G and δ_S are the isotopic composition of the total CO_2, added glucose and SOC, respectively.

Assumptions regarding the normality and homogeneity of the variances were checked, and the soil R_h and microbial biomass were natural log-transformed where necessary. The student's *t*-test was used to detect the influences of soil sieving and glucose addition on the soil R_h in each forest and the significant differences between mycorrhizal fungi types. The response of the soil microbial groups to sieving or glucose addition were calculated as the ratio of R_h in sieved soils to R_h in intact soils or the ratio of R_h in soils with glucose addition to R_h in soils without glucose addition, respectively. The relationships between the soil R_h, soil nutrients and microbial properties were assessed by Pearson's correlation analysis. The significance was at the probability level of $p < 0.05$, and the analyses were conducted using SPSS 19.0 for Windows. To further explore the relative importance of factors influencing the soil R_h, the random forest model analysis was conducted using the randomForest package in R 3.3.3 with default parameters [32].

3. Results

3.1. Responses of R_h to Soil Sieving and Glucose Addition

When no glucose was added across all forests, the average R_h was 5.94 mg C kg^{-1} soil d^{-1} and 4.03 mg C kg^{-1} soil d^{-1} in sieved soils on the 5th and 27th day of incubation, and 6.11 and 4.82 mg C kg^{-1}soil d^{-1} in intact soils, respectively (Figure S1). No significant difference in R_h between sieved and intact soils was observed in each forest (Figure 1). Glucose addition significantly affected the R_h in the *C. mollissima* and *P. massoniana* forests on the 5th and 27th day of incubation (Figure 1a,b,e,f) and in the *C. lanceolata* forest on the 27th day of incubation (Figure 1h). After the glucose addition, the R_h in the sieved soils was 28.0% and 26.3% lower than that in intact soils in both of the ECM forests on the 5th day of incubation (Figure 1a,b), and was 22.1% lower than that in intact soils in the *P. massoniana* forest (Figure 1e) but 78.0% higher in the *C. mollissima* forest on the 27th day of incubation (Figure 1f). However, glucose addition had no effect on soil R_h in the two AM forests (i.e., the *S. superb* and *C. lanceolata* forests) (Figure 1c,d,g,h). The above results suggest that glucose addition modified the influence of soil sieving on R_h in the ECM forests, but not in the AM forests.

When pooling all of the data together, significant and strong correlations between R_h in sieved and intact soils were observed ($r = 0.888$, $p < 0.01$) (Figure 2a). Similarly, the soil R_h in soils with and without glucose addition had significant correlations ($r = 0.826$, $p < 0.01$) (Figure 2b), and most data points were above the 1:1 line, suggesting that glucose addition significantly increased soil R_h.

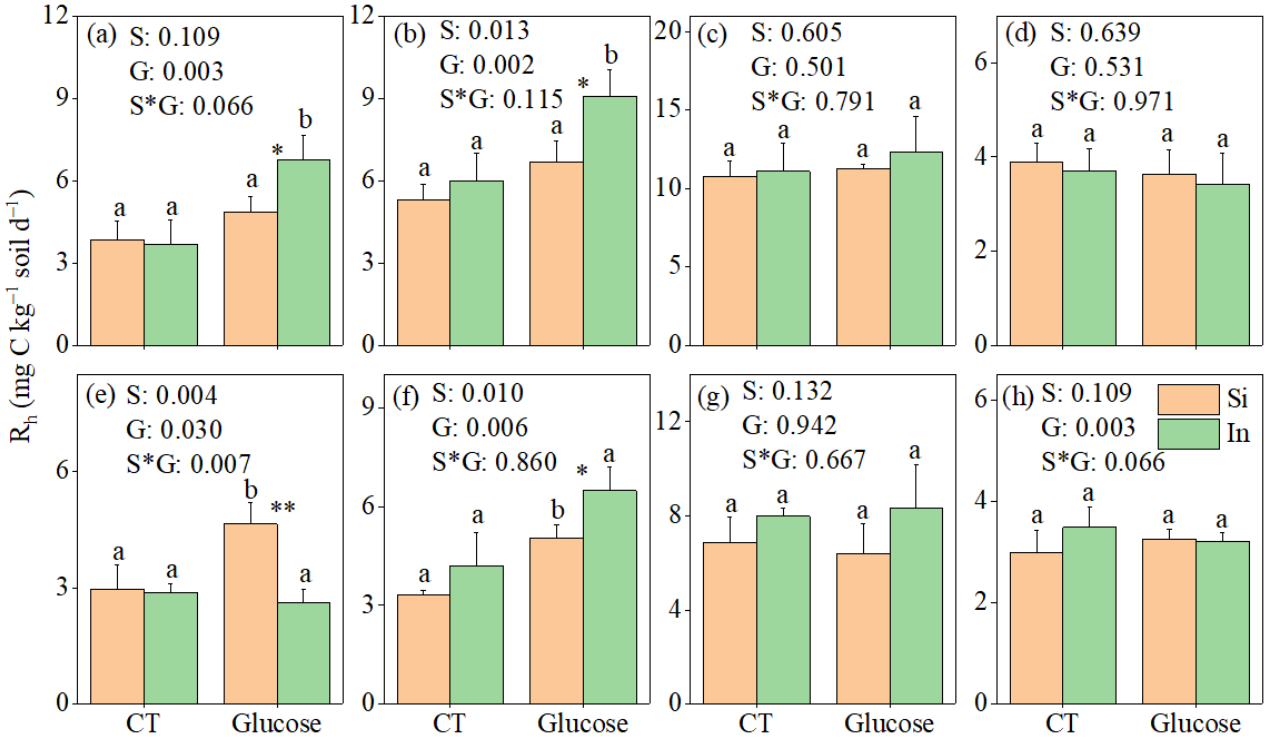

Figure 1. Effects of soil sieving(S) and glucose addition (G) on soil heterotrophic respiration (R_h) on the 5th (**a**–**d**) and 27th day (**e**–**h**) of incubation in four forests. Data flowing S, G and S*G were at the significance level (*p* values). a and e for *C. mollissima*, b and f for *P. massoniana*, c and g for *S. superba*, and d and h for *C. lanceolata* forests. Error bars denote standard deviation (n = 3). Different letters on bars denote significant effects of glucose addition on R_h for sieved or intact soils, and the asterisk *, ** denotes significant effects of sieving on R_h for CT or glucose-added soils at $p < 0.05$, 0.01, respectively.

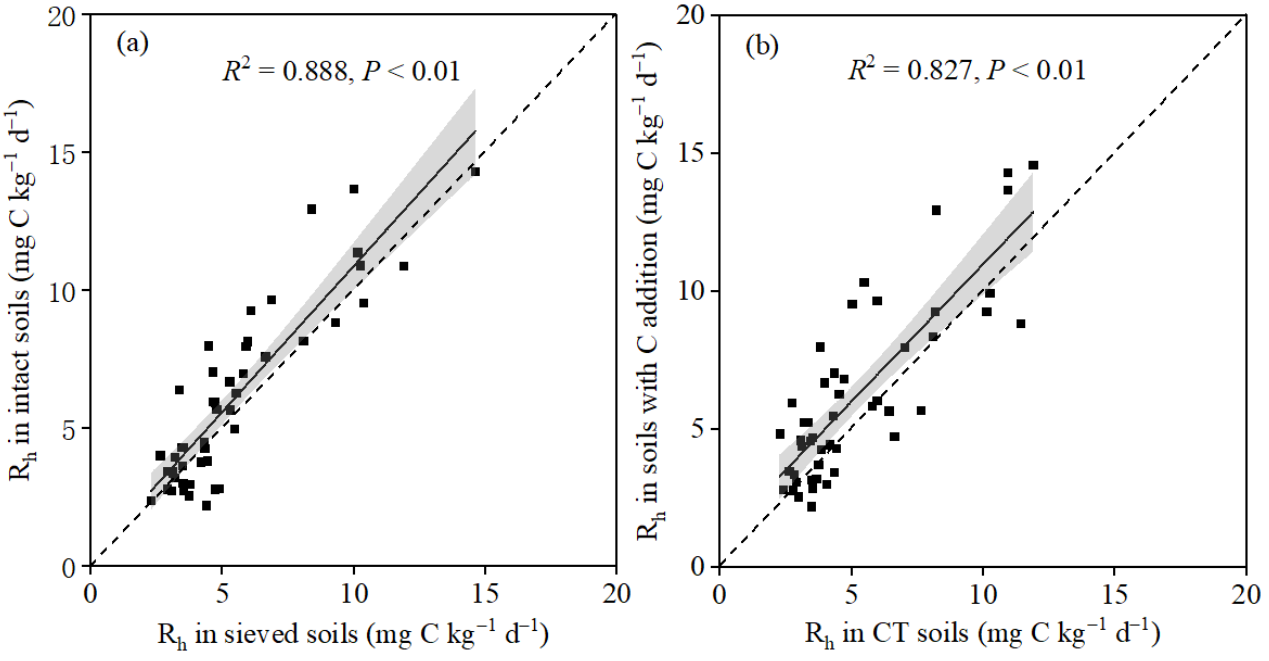

Figure 2. The correlation of soil heterotrophic respiration (R_h) between intact soils and sieved soils, (**a**) and between soils with glucose addition and soils without glucose addition (CT) (**b**) in forests. Grey shading represents the 95% confidence interval (n = 48).

3.2. Soil Microbial Responses to Sieving and Glucose Addition

In soils without glucose addition, sieving had less effect on the soil microbial biomass and community composition on the 5th day of incubation (Figure 3a), but significantly increased soil microbial biomass on the 27th day of incubation (Figure 3b). Sieving had increased the biomass of Ascomycota & Basidiomycota, Zygomycota and fungi than the other PLFA groups, but less modified the GN:GP ratio. The influences of soil sieving on microbial traits were regulated by glucose addition on the 5th day of incubation (Table 2), showing that glucose addition decreased the degree of the effects of sieving on soil microbial biomass (Figure 3). The results of two-way ANOVA demonstrated that the mycorrhizal fungi type and glucose addition had interactive effects on the ratio of PLFAs in sieved soils to that in intact soils. This demonstrated that glucose addition significantly increased the soil microbial biomass measured by PLFAs in both sieved soils and intact soils on the 5th day of incubation (Figure 4). However, on the 27th day of incubation, glucose addition tended to decreased microbial biomass in the sieved soils, but increased the microbial biomass in intact soils. The microbial community composition (e.g., F/B ratio) in sieved and intact soils also had different responses to glucose addition.

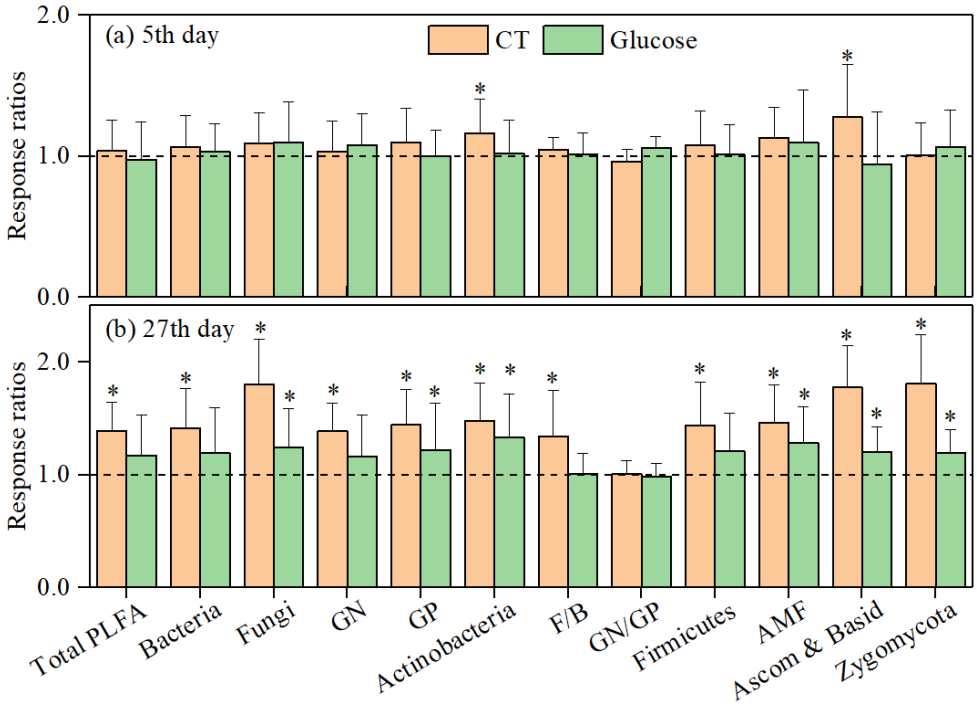

Figure 3. Effects of sieving on soil microbial biomass with and without glucose addition in four forests, as indicated by response ratios of the microbial biomass of different PLFA groups in sieved soils to that in intact soils on the 5th (**a**) and 27th day (**b**) of incubation. Error bars denote standard deviation (n = 12). Asterisk (*) on the bars denotes significant effects of sieving on soil microbial biomass at $p < 0.05$.

Table 2. Results (p values) of two-way ANOVA for soil sieving and glucose addition on soil microbial biomass on the 5th and 27th day of incubation in four forests.

Day		Total PLFA	Bacteria	Fungi	GN	GP	Actinobacteria	F/B	GN/GP	Firmicutes	AMF	Ascomycota & Basidiomycota	Zygomycota
5th	MFT	0.233	0.094	0.318	0.083	0.137	0.101	0.689	0.266	0.229	0.731	0.839	0.192
	Glucose	0.642	0.790	0.972	0.797	0.504	0.398	0.739	0.175	0.664	0.875	0.775	0.699
	MFT × G	0.011	0.033	0.034	0.042	0.033	0.273	0.355	0.944	0.019	0.237	0.973	0.033
27th	MFT	0.038	0.096	0.180	0.238	0.056	0.103	0.940	0.042	0.037	0.230	0.523	0.063
	Glucose	0.345	0.418	0.293	0.410	0.428	0.800	0.436	0.772	0.364	0.666	0.713	0.168
	MFT × G	0.944	0.883	0.622	0.955	0.727	0.800	0.486	0.297	0.803	0.820	0.612	0.408

MFT and glucose represent mycorrhizal fungi type and glucose addition, respectively.

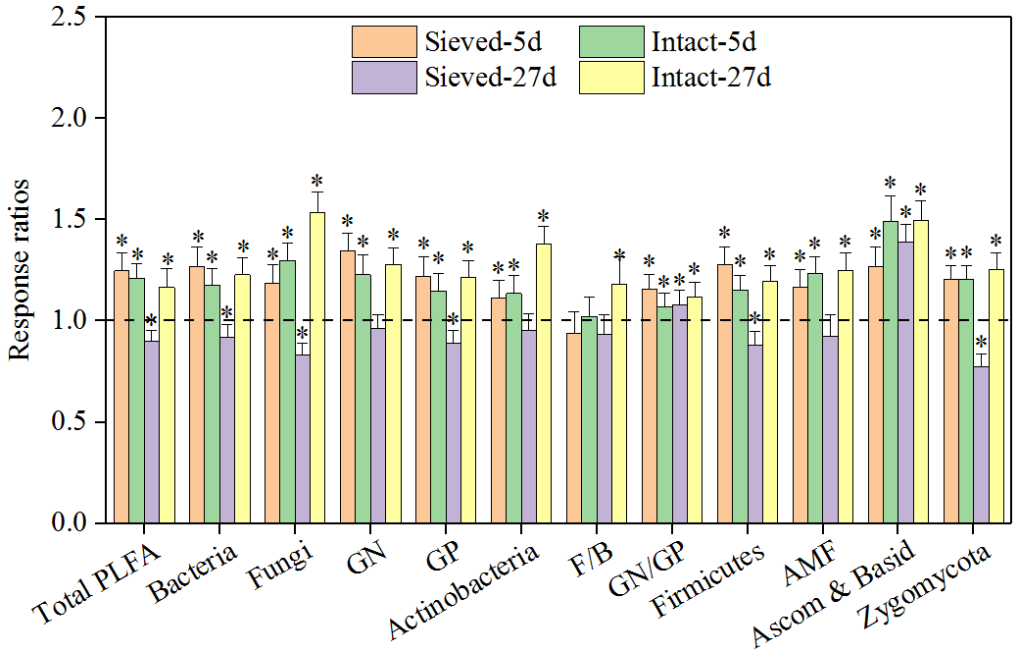

Figure 4. Effects of glucose addition on soil microbial biomass in sieved and intact soils in four forests, as indicated by response ratios of microbial biomass in soils with glucose addition to that in soils without glucose. Error bars denote standard deviation (n = 12). Asterisk (*) on the bars denotes significant effects of glucose addition on soil microbial biomass at $p < 0.05$.

3.3. Mechanism of Regulating Soil R_h

The results of the correlation analysis showed that R_h was strongly and positively correlated with the biomass of various microbial groups measured using the PLFAs on the 5th and 27th day of incubation. However, it was not related to the ratios of fungi to bacteria and gram-negative to -positive bacteria (Figure 5). Their correlation coefficients became lower on the 27th day (Figure 5b) than the 5th day (Figure 5a). In addition, the soil R_h was strongly and positively correlated with mineral N, and negatively correlated with available P. We further conducted the random forest model analysis, and the results showed that the measured soil variables explained 74.7% and 49.7% of the variation in soil R_h on the 5th and 27th day. On the 5th day of incubation, the total microbial and firmicute biomasses were more important in regulating soil R_h than the other PLFA groups, and available P was also important (Figure 6a). The available P was the most important, and the biomass of Ascomycota, Basidiomycota and mineral N was also more important on the 5th day of incubation (Figure 6b). These results suggested that the roles of soil nutrients and microbial PLFA groups in regulating soil R_h varied temporally.

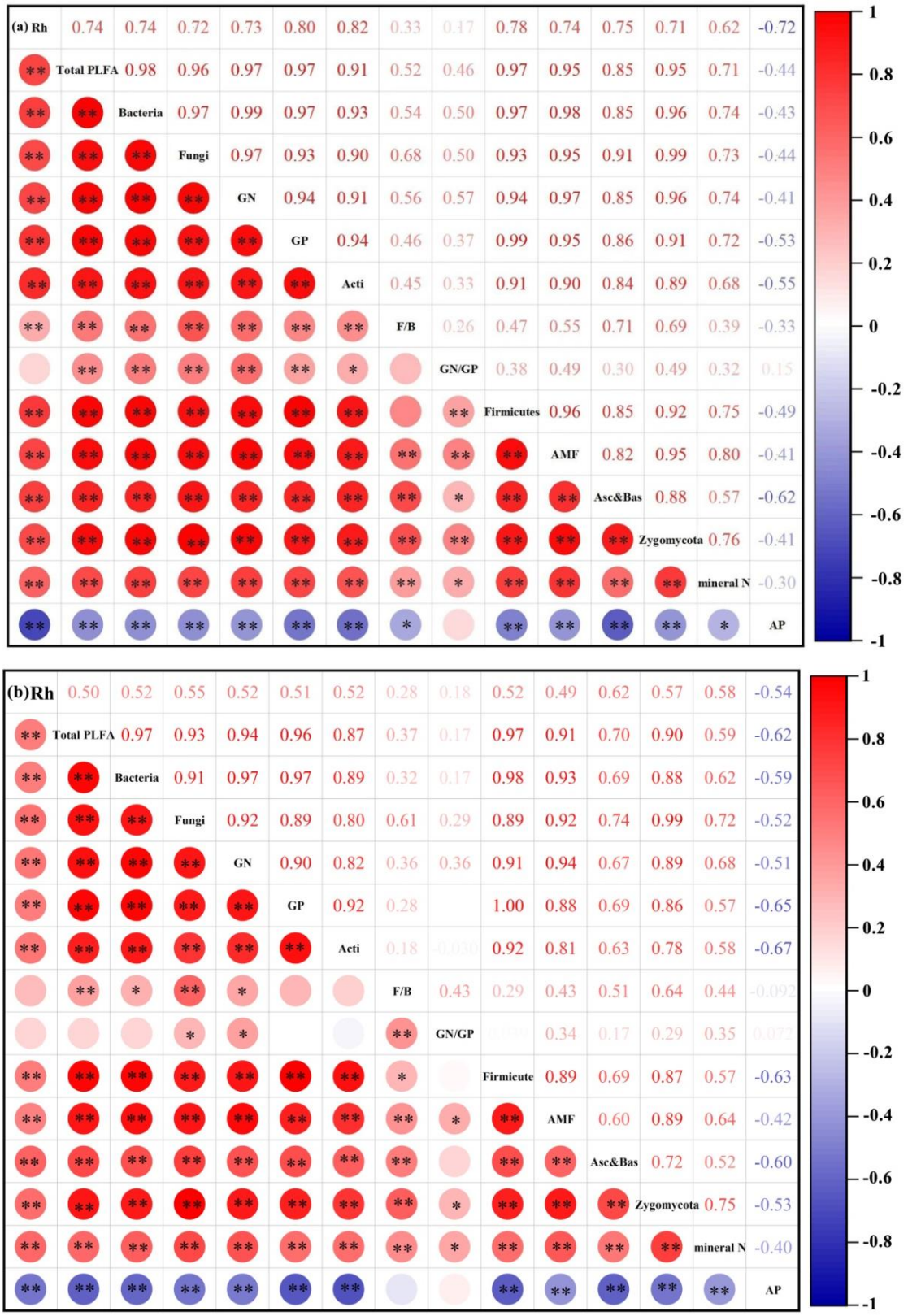

Figure 5. Correlations of soil heterotrophic respiration in sieved and intact soils to soil available nutrients and microbial properties onthe 5th (**a**) and 27th day (**b**) of incubation. *, ** denote significance at p = 0.05 and 0.01, respectively.

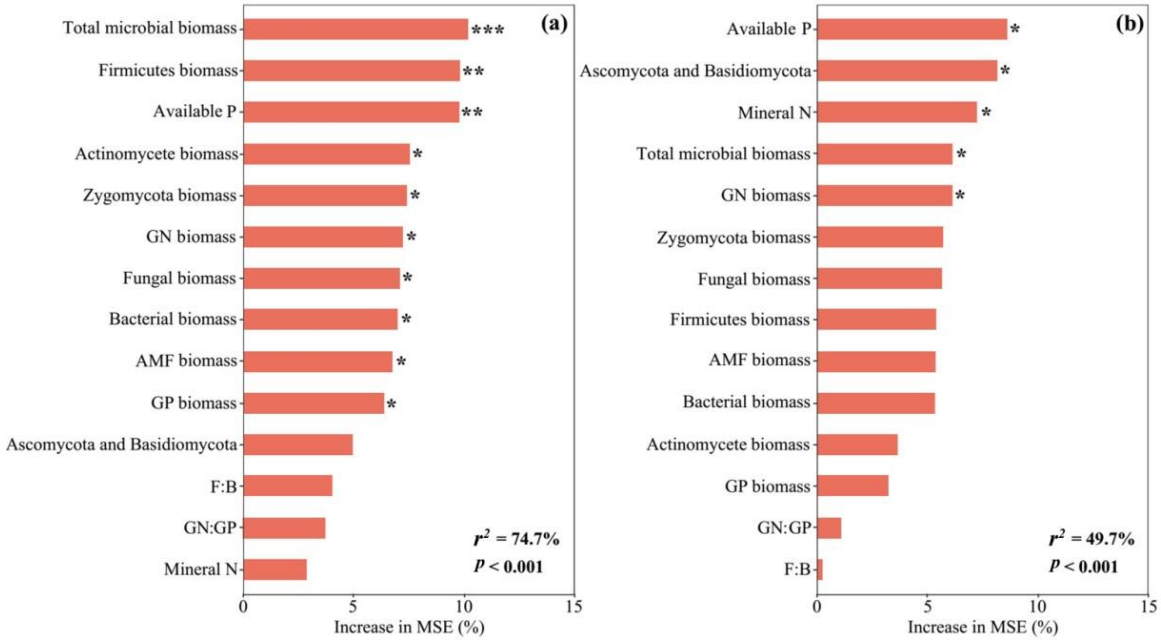

Figure 6. Main variables explaining soil heterotrophic respiration on the 5th (**a**) and 27th day (**b**) of incubation according to random forest model analysis. *, **, *** denote significance at p = 0.05, 0.01, 0.001, respectively.

4. Discussions

Although sieving is an important pretreatment practice for most laboratory incubation experiments, the influence of sieving on soil R_h or SOC decomposition is less clear, especially with regards to what microbial factors are controlling its effects. Compared to previous studies [12,14,17,18], our study was the first to explore how C availability regulates the impacts of sieving on soil R_h. We found that sieving had less influence on R_h, but that glucose addition changed the influence of soil sieving on soil R_h in the ECM forests but not in the AM forests. Furthermore, the roles of soil nutrients and microbial PLFA groups in regulating soil R_h varied temporally.

4.1. Response of Soil Heterotrophic Respiration to Sieving

Unlike our expectation that sieving would promote soil R_h, no significant difference in soil R_h between sieved and intact soils in the four forests was observed when no glucose was added into the soils during incubation (Figures 1 and S1), suggesting that sieving has no effect on soil R_h. This result was consistent with previous experiments that found no significant influence of soil sieving on R_h or SOC decomposition [11,17,18,33,34], although some experiments found sieving stimulated a short-term CO_2 flux [12,14,15,35]. First, these conflicting observations were in part explained by the methodological differences such as the mesh size used for soil sieving. Černohlávková et al. [36] reported that the effects of sieving on soil respiration and microbial biomass C were related to mesh size in arable, grassland and forest soils, and higher soil respiration was observed in soils sieved through the finer mesh [15,35]. This indicated that the sieve mesh size affected the release of labile organic matter such as carbohydrates, because soil sieving through a smaller mesh demands more force and the soil's physical structure is disrupted [37]. In our study, the lesser effect of sieving on soil R_h was related to a large mesh size (e.g., 2 mm). Another potential reason was that the influence of sieving on soil R_h was dynamically changed with the incubation time. The effect of sieving on soil R_h appeared to last a few days (e.g., [38]), and was greater at the early stage of incubation rather than the later stage [39]. In our study, sieved soils were pre-incubated for 7 days, which may reduce the effects of soil sieving on R_h.

4.2. Carbon Addition Mediating the Response of Soil Heterotrophic Respiration to Sieving

To our knowledge, this was the first study to explore how increasing C availability affects the response of R_h to sieving. We found that glucose addition caused lower R_h in sieved soils than intact soils in the ECM forests (i.e., *C. mollissima* and *P. massoniana*) but not in the AM forests on the 5th day of incubation (Figure 1a,b), which was different from our hypothesis that glucose addition would decrease the stimulatory effects of sieving on soil R_h. We also found at the later incubation stage (i.e., on the 27th day of incubation) that glucose addition had an opposite influence on the responses of R_h to soil sieving. It showed lower R_h in the sieved soils than in the intact soils in the *P. massoniana* forest (Figure 1e), but higher in the *C. mollissima* forest (Figure 1f) on the 27th day of incubation. These findings suggest that the influence of glucose addition on the response of R_h to soil sieving had a mycorrhizal type-specific in forest ecosystems. As per our observation (Figures 2b and S1), many studies found that carbon addition accelerated soil R_h or SOC decomposition in laboratory incubation experiments [8,13,24,25,40]. This phenomenon is usually defined as a priming effect [41], suggesting that C availability is a primary factor in limiting microbial processes. The stimulatory effect of C addition was in part explained by the microbial nutrient mining hypothesis that soil microorganisms can utilizelabile C as an energy source to decay SOC and acquire nutrients [24,42]. A decrease in the mineral N (NH_4-N and NO_3-N) and available P concentrations after glucose addition during incubation, especially on the 5th day (Figure S2), supported this opinion.

The microbial stoichiometric decomposition theory also explained why C addition accelerated R_h in intact soils in the ECM forests. Glucose addition did not match microbial demands for C, N and P, that is, this input unbalanced the microbial stoichiometric C, N and P ratios. Therefore, glucose addition accelerated soil organic matter decomposition by the domination of r-strategists [43]. This was confirmed by the increased GN:GP ratio (Figure 4) because gram-negative bacteria preferentially decompose labile substrates, whereas gram-positive bacteria are able to utilize more complex substrates [44]. This was supported by our results that the mineral N and available P strongly related to soil R_h (Figure 5), and were important factors in regulating soil R_h (Figure 6). This was in agreement with some nutrient addition experiments that the availability of N and P plays important roles in organic matter decomposition in tropical soils [8].

Different responses of soil microbial biomass and community composition (e.g., F/B ratio) were observed in sieved and intact soils to glucose addition (Figure 4; Table 2). They were also responsible for the modification of glucose addition to soil sieving on R_h in ECM forests, because soil microbes are the primary drivers of soil R_h [19–21]. Glucose addition tended to decrease the soil microbial biomass in the sieved soils, but increased the soil microbial biomass in the intact soils on the 27th day of incubation (Figure 4), suggesting that glucose addition changed the responses of soil microbial biomass to sieving. Sieving soil increased microbial biomass on the 27th day of incubation, in particular in soils without glucose addition (Figure 3). This was consistent with some previous observations [45,46], but fewer studies assessed their responses to glucose addition in intact soils. The increase of soil microbial biomass in the sieved soils could be explained by an increase in C availability, resulting from a decrease in the aggregate protection for SOC [11,13,47] and the changes in soil microbial community and functional diversity after sieving [46].

In the present study, although some important findings were discovered, we noted that our results should be applied in other forests or regions with caution. Our findings had some implications for future research on this issue. Firstly, we added glucose to simulate the carbon input via plant roots, but root exudates are complex and include numerous materials such as sugars, amino acids and organic acids [48]. That is to say, results from added glucose in this experiment may have some differences to the results occurring in the field. Thus, the composition of added labile substrates should be the same as root exudates and/or litter in a future study. Secondly, some studies showed that the effects of sieving on soil respiration were dependent on the mesh size [15,35,36], and the effects of sieving on soil respiration varied over incubation (Figure 1) [40], suggesting that it is critical to

choose the incubation duration and sampling time. Therefore, formulating an experimental framework is important in order to understand the effects of soil sieving on soil respiration. Thirdly, only four forest types were investigated and we found that in ECM forests, glucose addition modified the effects of sieving on soil respiration. However, to obtain a general and solid conclusion, similar experiments should be widely conducted in other biomes in future.

5. Conclusions

Our study explored how sieving affected soil R_h, and we found the effect of soil sieving on R_h was regulated by C addition in forest ecosystems. Our results demonstrated that sieving had less of an influence on soil R_h, but that glucose addition made this influence significant in two ECM forests but not in two AM forests. This suggests that the functional plant type (e.g., mycorrhizal type) influenced the roles of the increase in C availability to microbes in regulating the response of the soil R_h to sieving. The imbalance caused by glucose addition between C and nutrients, in particular N, changed the responses of the soil R_h to sieving by altering the soil microbial biomass and community in the ECM forests. The significant and strong correlations between sieved and intact soils for R_h indicate that sieved soils are suitable to evaluate the relative influence of forest types or management practices on soil respiration in forest ecosystems. These findings somewhat enhanced our understanding of the response of R_h to soil sieving in increasing C-input scenarios in terrestrial ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13081263/s1, Figure S1: Average effects of soil sieving and glucose addition on heterotrophic soil respiration (R_h) in forests. Error bars denote standard deviation (n = 12); Figure S2: Effects of glucose addition on soil nutrients in all soils, sieved soils and intact soils as indicated by response ratios of nutrients in soils with glucose addition to that in soils without glucose. Asterisk on the bars denotes significant effects of glucose addition on soil nutrients at $p < 0.05$. Error bars denote standard deviation (n = 12). Table S1: Assignment of phospholipid acids to different main microbial groups according to the method of Joergensen (2022).

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