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ARTICLE

Mitigating N_2O emissions from agricultural soils with fungivorous mites

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Abstract

Nitrous oxide (N_2O) is an important greenhouse gas and an ozone-depleting substance. Due to the long persistence of N_2O in the atmosphere, the mitigation of anthropogenic N_2O emissions, which are mainly derived from microbial N_2O-producing processes, including nitrification and denitrification by bacteria, archaea, and fungi, in agricultural soils, is urgently necessary. Members of mesofauna affect microbial processes by consuming microbial biomass in soil. However, how microbial consumption affects N_2O emissions is largely unknown. Here, we report the significant role of fungivorous mites, the major mesofaunal group in agricultural soils, in regulating N_2O production by fungi, and the results can be applied to the mitigation of N_2O emissions. We found that the application of coconut husks, which is the low-value part of coconut and is commonly employed as a soil conditioner in agriculture, to soil can supply a favorable habitat for fungivorous mites due to its porous structure and thereby increase the mite abundance in agricultural fields. Because mites rapidly consume fungal N_2O producers in soil, the increase in mite abundance substantially decreases the N_2O emissions from soil. Our findings might provide new insight into the mechanisms of soil N_2O emissions and broaden the options for the mitigation of N_2O emissions.

Introduction

Nitrous oxide (N_2O) is a greenhouse gas with a global warming potential that is ~300 times higher than that of an equivalent concentration of carbon dioxide (CO_2) [1]. N_2O is also an important ozone-depleting substance (ODS) [2]. Currently, the ozone depletion potential-weighted emissions of N_2O are greater than those of any of the ODSs controlled under the Montreal Protocol on Substances that Deplete the Ozone Layer [2]. Less than 1% of atmospheric N_2O is naturally removed annually, and this removal mainly occurs by photolysis and oxidative reactions in the stratosphere [2], which has resulted in the accumulation of N_2O from a preindustrial concentration of 270–319 ppb in 2005 [3]. Given the pressure to keep global warming to 1.5 °C above the preindustrial levels [4] and to address ozone depletion, the mitigation of anthropogenic N_2O emissions has become an urgent task. Agricultural activity accounts for more than half of the global anthropogenic N_2O emissions and thus offers great potential for N_2O mitigation [5–7].

N_2O emitted from agricultural soils is primarily derived from microbial processes in soil following the application of nitrogen fertilizer, and these processes include nitrification and denitrification by bacteria, archaea, and fungi [1, 8–10]. Nitrification is the process through which NH_4^+ (or NH_3) is oxidized to NO_3^- via NO_2^-. N_2O is formed during the oxidation of NH_3 to NO_2^- by ammonia-oxidizing bacteria or archaea [11]. N_2O is also formed as an intermediate during denitrification by bacteria, which involves the stepwise reduction of NO_3^- to N_2 via NO_2^-, NO, and N_2O [11]. Fungi are also capable of denitrification [12]. Unlike denitrifying bacteria, all known denitrifying fungal isolates produce N_2O as the end product of denitrification due to the lack of nitrous

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oxide reductase, which catalyzes the reduction of N_2O to N_2 [11, 12]. Studies on denitrification in agricultural soils have historically focused on bacterial denitrification, but recent studies have demonstrated that denitrification is a widespread trait in fungi [13–16], and the contribution of fungal denitrification to N_2O emissions cannot be ignored [15–19]. Fungal denitrification reportedly dominates N_2O emissions under specific conditions in various soils, including grassland soils [17], forest soils [18], coastal sediments [19], and agricultural soils [15]. In particular, in cropland whose surface has been applied with organic fertilizer, fungal denitrification could account for more than 80% of N_2O emissions [15], probably because the fungal biomass is generally high in soils with a high organic matter content and fungal denitrification preferentially occurs over bacterial denitrification under microaerophilic conditions [15, 20].

Soil microbes are involved in trophic interactions with soil fauna, and thus, many microbial processes in soil are affected by the feeding activities of soil fauna [21]. Soil mesofauna, as the group of soil fauna with a size of 0.2–2 mm, comprise the dominant fungivores in soil because most mesofauna members (mites, springtails, etc.) feed on fungi [21]. Fungivorous mites, one of the most abundant soil mesofaunal groups, mainly consist of most Oribatida, Astigmata, and a partial species of Prostigmata [22]. These mites populate soils in various terrestrial ecosystems, including forests, grasslands [22], and even deserts [23]. Fungivorous mites also inhabit most agricultural soils [24–26], and their population is often disturbed by agricultural practices such as fertilization, pesticide/herbicide application, tillage [24, 26], and irrigation [27]. Fungivorous mites are known to play important roles in N mineralization by feeding on fungi [28]. Some recent lab-scaled studies have suggested that some fungivorous mites (suborder Astigmata), as well as other mesofauna members (Enchytraeids), could be involved in the N_2O dynamics due to the shift in the nutrient availabilities in soil resulting from their effect on N mineralization [29, 30]. Because many fungivorous mites are generalists that can indiscriminately feed on diverse fungal taxa [22] and N_2O production is a widespread trait in fungi [13–15], it is probable that fungivorous mites not only indirectly affect N_2O emissions via their influence on nutrient availability but also directly consume the biomass of N_2O-producing fungi in soil to some extent and are therefore even related to the N_2O emissions derived from fungal denitrification. However, the related empirical evidence is scarce: (1) although the general fungal feeding habits should cover N_2O-producing fungi, there is a lack of data on the feeding behaviors of mites toward known N_2O-producing fungal strains; and (2) although fungal consumption by fungivorous mites is known to be common in agricultural soils, whether the consumption is sufficiently intensive to change the fungal biomass after fertilization, which usually relies on the relative abundance of mites to fungi, remains unclear [21].

In the current study, we aimed to test the hypothesis that effectively increasing the numbers of fungivorous mesofauna can mitigate N_2O emissions from agricultural soils by taking advantage of the fungal feeding ability of mesofauna. This hypothesis was generated internally based on the fortuitously observed finding that the addition of coconut husks (the dried husks of coconut fruits are a low-value part of coconut and are commonly employed in agriculture as a soil conditioner because its porous structure can improve the air and water permeability of soil [31, 32]) to soil in a cropland field resulted in reduced N_2O emissions (first field experiment). Because we found that numerous fungivorous mites lived in the space between the fibers of coconut husks and because it was previously suggested that factors contributing to an increase in the soil invertebrate abundance appear to reduce the biomass of saprotrophic mycelia and consequently decrease fungal-mediated soil processes [33], we hypothesized that the reduction in N_2O emission observed after the application of coconut husks was mediated by fungivorous mites through a process consisting of three steps (Fig. 1): (1) coconut husks increase mite abundance in soil by housing mites within their porous structure, which might be a favorable habitat for mite reproduction (hypothetical step 1); (2) the mites, whose number has increased, decrease the abundance of denitrifying fungi in soil by feeding intensively on denitrifying fungi (hypothetical step 2); and (3) the decrease in the abundance of denitrifying fungi causes a decrease in the N_2O emissions from soil (hypothetical step 3). We performed another field experiment (second field experiment), a series of microscopic observations, and two soil microcosm experiments (first and second microcosm experiments) to verify the hypothesis. To verify the effect of coconut husk application on mite abundance (hypothetical step 1), we performed a second field experiment and a first microcosm experiment and determined the shifts in the mite abundances after coconut husk application. In addition, if hypothetical steps 2 and 3 are correct, mites should affect N_2O emissions independent of the presence of coconut husks, and conversely, the effect of coconut husks on N_2O emissions should rely on the activities of mites. Therefore, we tested the effect of mites on N_2O emissions by manually introducing mites to mite-free soil microcosms (first microcosm experiment) and assessed the effect of coconut husks on N_2O emissions by controlling the mite abundance through the use of a miticide in the field (second field experiment) or in mite-free soil microcosms (second microcosm experiment). Furthermore, we directly verified the effect of an increased mite abundance on the abundance of denitrifying fungi (hypothetical step 2) and tested whether the effect was obtained due to their feeding behaviors against denitrifying fungi. To that

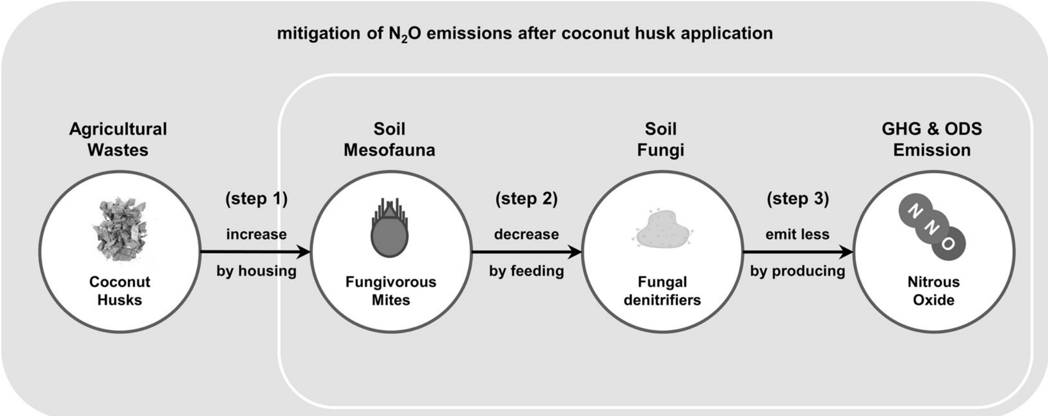


Fig. 1 Hypothesis investigated in this study. We hypothesized that the N_2O mitigation process after coconut husk application consists of three steps: (1) coconut husks increase the mite abundance in soil by housing mites within their porous structure, which might be a favorable habitat for mite reproduction; (2) the mites, whose number has increased, decrease the abundance of denitrifying fungi in soil by intensively feeding on these fungi; and (3) the decrease in the abundance of denitrifying fungi causes a decrease in N_2O emissions from soil.

end, we observed the feeding habits of the mites on soil surface grown with mycelia and on agar plates grown with various pure cultures of denitrifying fungal strains and quantified the shift in the abundance of denitrifying fungi after introduction of the mite to the soils in the second microcosm experiment. In the microcosm experiment, the shift in N_2O emissions was also determined to test the effect of the decrease in the abundance of denitrifying fungi on N_2O emissions (hypothetical step 3).

Methods

Experimental field setup

The experimental field was located in Nagaoka city, Niigata Prefecture, Japan (N37°26′, E138°52′). The soil is an Andisol, which is the most widespread upland agricultural soil type in Japan [34]. Its average physical–chemical properties are as follows: total carbon, 39 g C/kg dry soil; total nitrogen, 2.6 g N/kg dry soil; bulk density, 0.81 g cm^3; solid-phase rate, 34.5%; and pH, 6.5 [15]. The fertilizer used was a commercially available granular organic fertilizer that comprises a mixture of food manufacturing residues, including feather meal, fish meal, rapeseed meal, rice bran, oil palm ash, and poultry litter ash (total N: 6%, P_2O_5: 6%, and K_2O: 6%). The same fertilizer has been used in several previous studies related to N_2O emissions from agricultural soils [15, 35–38].

First field experiment

The first field experiment, in which the effect of coconut husk application on N_2O emissions after fertilization was tested, was performed in the spring of 2016. The experiment was set up in an area of ~7 × 14 m in the experimental field (Fig. 2a). The field was divided into 12 plots (1.7 × 2.9 m) and randomly assigned to two treatments (complete randomized design, Fig. S1a, b): six plots were only applied with the fertilizer as a control (F), whereas the other six plots were applied with both the fertilizer and coconut husks (FH).

Basal fertilization (30 g N/m^2) was performed on May 18, 2016, in all the plots by mixing the fertilizer into the 0–10-cm layer of the soil. The coconut husks were also mixed into the 0–10-cm layer of the soil together with the basal fertilizer in half of the plots (FH). The amount of coconut husks applied to the soil was 45 g/m^2, and the volume ratio of coconut husks to soil was ~1%. The coconut husks were applied in the form of chips a few centimeters in size prepared from dried husks of coconut fruit (Fig. 2b) and were purchased from Fujick Co., Ltd., Tokyo, Japan. The sweet corn seeds were then sown in all the plots, and nine strains (~3.6 strains/m^2) were planted in each plot. The sweet corn used in this experiment was of a variety called "Gold Rush" (*Zea mays L.* var. *saccharata*). Supplemental top-dressing fertilization (20 g N/m^2) was performed on June 8, 2016, in all the plots. The sweet corn was harvested on August 8, 2016.

The N_2O flux in the field was determined every week using the chamber method (Fig. S1b–d). The details of the method are described elsewhere [15]. The daily precipitation and daily temperature were recorded every day by a weather station of the Japan Niigata Agricultural Institute (NASCON 2000, Yokogawa Weathac Corporation, Japan). The soil moisture (water-filled pore space) was measured every day by ECH_2O EC-5 sensors coupled with an automatic datalogger (METER Group, Inc., USA) set in the field. The ears of the sweet corn were weighed with a balance. For better measurement accuracy, the husks and stem were removed before weighing. The

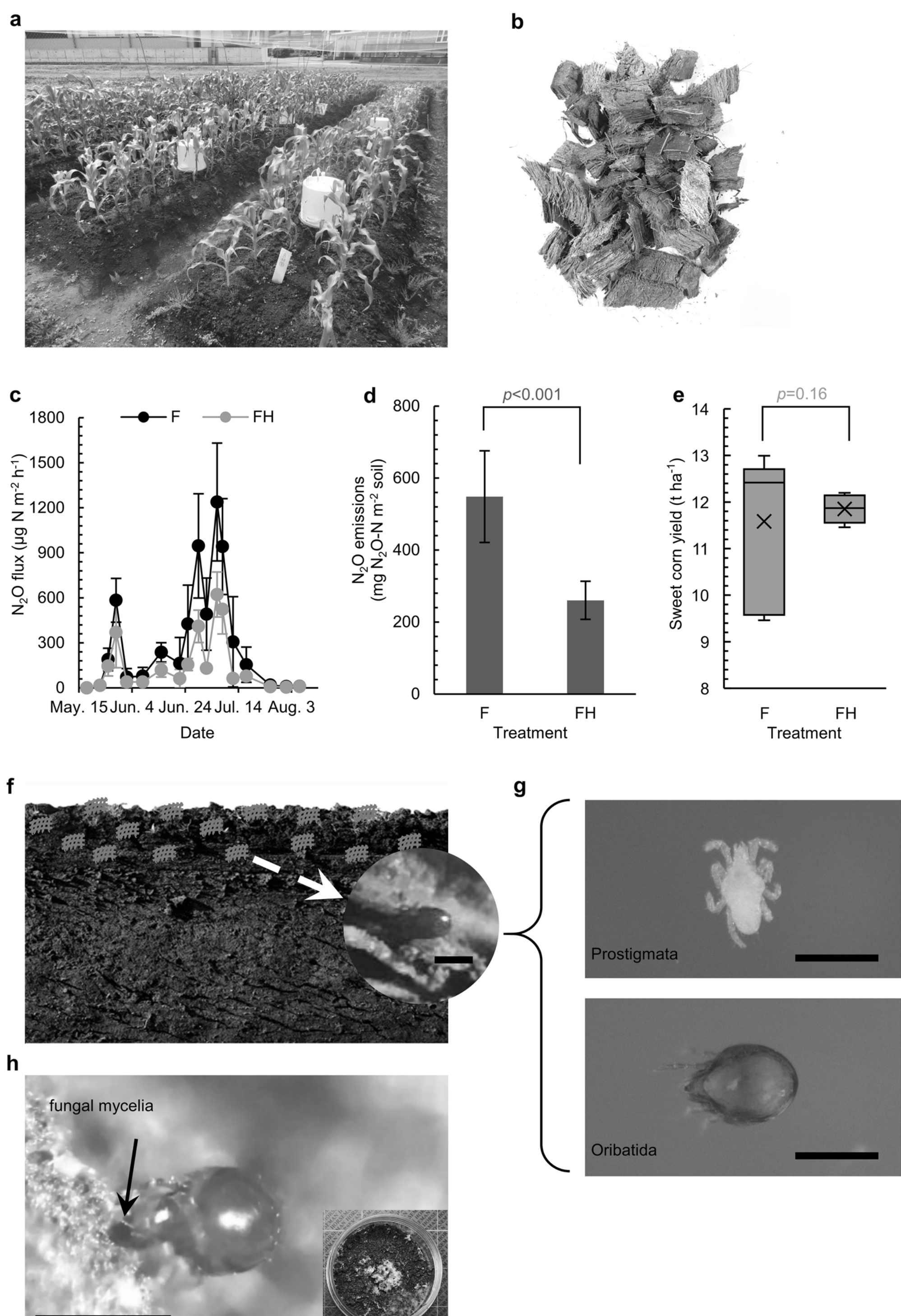


Fig. 2 Mitigation of N_2O emissions from soils in a sweet corn field after coconut husk application. **a** Experimental field planted with sweet corn. The white chambers were used for gas sampling to determine the N_2O flux. **b** Coconut husks applied to the experimental field. The bar indicates 2 cm. N_2O fluxes (**c**) and cumulative N_2O emissions (**d**) during the cultivation period. F: soils applied with fertilizer only; FH: soils applied with fertilizer and coconut husks. The data are plotted as the means ± SDs ($n = 6$). **e** Yield of sweet corn. The crosses indicate the mean values from replicates ($n = 6$). The bars indicate the standard deviations from the replicates ($n = 6$). The upper and lower ends of the boxes indicate the upper and lower quartiles, respectively; the line within the box indicates the median. F: soils applied with fertilizer only; FH: soils applied with fertilizer and coconut husks. **f** Mites living in the gaps of coconut husks. **g** Major mite taxa found in the coconut husks. **h** Mites feeding on fungal mycelia growing on soil particles. The bars indicate 0.3 mm (**f**–**h**). In all figures, p values were calculated by independent-sample t-tests (assuming equal variances, two tailed) and are indicated in the figures.

yield of sweet corn was calculated in kilograms per hectare (kg/ha, 1 ha = 10^4 m^2).

Observation of mites in coconut husks and their feeding habits

After the first field experiment, a small number of the applied coconut husks were sampled from the field for stereomicroscope observation. The coconut husks were slightly tapped to remove the residual soil, placed on a Petri dish, and observed under a stereomicroscope. During the observation, the husks were gradually teared along the fibers to observe their insides. To determine whether the mites found in the coconut husks sampled from the field (see "Results" subsection "The application of coconut husks decreases N_2O emissions, and fungivorous mites are observed in the coconut husks") originally inhabited the coconut husks prior to their application to the field or immigrated from the field soil, mites were extracted from 400 g of newly unpacked coconut husks and field soils that had never been treated with coconut husks (collected from F in the first field experiment) using a Tullgren apparatus. The details of the method used for extracting mites using the Tullgren apparatus are described elsewhere [39]. The mites found in the coconut husks were identified by combining information on their morphological properties and their DNA sequence (partial region of the 18S rRNA genes). The details are available in the Supplementary Information (Supplementary Methods 1).

To preliminarily test whether the mites are fungivores, dozens of grams of field soil was collected into a small vial, several granular organic fertilizers (the same fertilizer as that applied in the experimental field) were applied to the surface, and the samples were incubated at 25 °C until fungal mycelia grew (~1 week). A dozen mites found in the coconut husks were then transferred to the soil surface using a small brush. The behaviors of the mites were immediately observed under a stereomicroscope. Videos and photographs were taken during the observation.

The feeding behaviors of the mites were further tested in vitro using pure cultures of N_2O-producing fungal strains. N_2O-producing fungi were isolated from the experimental field soil (details of the isolation of N_2O-producing fungal strains are described in Supplementary Methods 2), inoculated onto the center of PDA plates and cultured at 25 °C for 3–7 days. The isolated individual mites were then transferred to PDA plates grown with mycelia. The behaviors of the mites were immediately observed under a stereomicroscope. Videos and photographs were taken during the observation.

Second field experiment

The second field experiment, in which the effect of coconut husk application on the mite population in the soil was quantified, was performed in the autumn of 2016. In addition, the effect of coconut husk application on N_2O emissions in the absence of mite activities was investigated by using a miticide to maintain the mite abundance at a low level. The experiment was set up in an area of ~3 × 10 m in the experimental field, and this area was located next to the area used for the first field experiment (Fig. S2). In this experiment, a crop was not planted in the field. A completely randomized design was utilized, and the field was divided into 12 replicated plots (1.3 × 1.3 m) with the following four treatments (Fig. S2): fertilizer alone (F), fertilizer plus coconut husks (FH), fertilizer plus miticide (FI), and fertilizer plus coconut husks and miticide (FHI).

To reduce the workload, the treatments including fertilization, coconut husk application, and miticide application were only performed within the area of the chamber base of the chamber apparatus for determination of the N_2O flux (Fig. S2). Because the treatments were only performed within the chamber base area and each chamber base was ~1 m away from another, the gaps between plots were omitted. Each replicate included two sets: one was used for gas sampling, and the other was used for destructive soil sampling (Fig. S2). Basal fertilization (37.5 g N/m^2) was performed on September 12, 2016, by mixing the fertilizer into the 0–10-cm layer of the soil (Fig. S3a). Supplemental top-dressing fertilization (15.0 g/m^2) was performed on September 30, 2016. The coconut husks were applied to the soil using the same method but at an amount that was 5.5-fold higher (250 g/m^2) than that used in the first field experiment to exaggerate the effects of the coconut husks (Fig. S3a). The miticide was applied to the soils by spray injection for 5 s after gas sampling each week (Fig. S3b). The miticide contained phenothrin (0.25 w/v%), metoxadiazone (0.025 w/v%), and amidoflumet (0.2 w/v%) and was commercially available (Earth Corporation, Tokyo, Japan).

The N_2O flux (Fig. S3c, d), daily precipitation, and daily temperature were measured using the same methods as in the first field experiment. Soil samples for determination of the mite abundance were obtained by destructive sampling 3 days prior to supplemental fertilization. The soil mites were isolated from the soil and coconut husks using a Tullgren apparatus [39]. 400 g of soil or soil containing coconut husks was placed in the Tullgren apparatus for 6 h (Fig. S3e, f), and the extracted mites were collected in a small vial with 70% ethanol. The number of mites was counted under a stereomicroscope.

Setup of soil microcosms

Soil microcosm experiments (Fig. S4a–c) were set up such that the mite abundance was controlled by manually introducing mites extracted from the field soil to mite-free soils. Mite-free soils were obtained by passing the soil collected from the

experimental field through a 2-mm mesh sieve and storing it at 4 °C in sealed plastic bags for more than 1 month. Compared with the use of miticide for controlling the mite abundance in the second field experiment, this method can avoid unexpected side effects on soil chemical properties or microorganisms in the soil during mite abundance control. The soil microcosms were set up in 650-ml glass bottles. Each bottle contained 200 g of soil (150 g in dry weight), and the soil moisture was adjusted to 60% of its maximum water holding capacity by adding distilled water. The fertilizer used was the same granular organic fertilizer used in the field experiments.

First microcosm experiment

In the first microcosm experiment (Fig. S4a), the effects of coconut husk application on the mite abundance and the relationship between the mite abundance and N_2O emissions were tested. For basal fertilization, 2 g of organic granular fertilizer was evenly mixed into the soil on the 1st day of incubation, and for supplemental fertilization, 5 g of organic granular fertilizer was applied to the surface of the soil on the 31st day of incubation. The microcosm experiment contained the following three treatments: application of fertilizer only as a control (F′), application of fertilizer plus mites (F′M), and application of fertilizer plus mites and 2 g dw of coconut husk chips (F′HM). The mites applied to the soil were obtained from the whole communities of mites extracted from 400 g of the field soil using the Tullgren apparatus. Each treatment contained three replicates. After their establishment, the microcosms were incubated at 25 °C. The N_2O flux was measured every 2–4 days following methods described elsewhere [15]. The mites were also extracted, collected, and counted using almost the same procedure as that used in the second field experiment. The mites were extracted from the soil and coconut husk chips in the microcosm experiment to investigate the mite abundance in both the soil and the husks. Soil samples and coconut husk samples were collected on the last day of the incubation for determination of the mite abundance. Half of the soil (75 g in dry weight) in the microcosm was used for the extraction of mites using the Tullgren apparatus. All coconut husks in the microcosm were used for the extraction.

Second microcosm experiment

In the second microcosm experiment (Fig. S4b, c), the shift in the abundances of fungi and fungal denitrifiers in soil and the shift in N_2O emissions after the introduction of mites to the mite-free soils were quantified. In addition, using the mite-free soils, the effect of coconut husks on N_2O emissions in the absence of mite activities was tested. 2 g of organic granular fertilizer was applied to the surface of the soil on the 1st day of incubation. The microcosm experiment contained three treatments: application of fertilizer only as a control (F′, seven replicates), application of fertilizer plus 80 Oribatida individuals (F′M, seven replicates), and application of fertilizer plus coconut husk chips (F′H, three replicates). After their establishment, the microcosms were incubated at 25 °C. The N_2O flux was measured every 2–4 days following methods described elsewhere [15]. Three of the seven replicates of the F′ and F′M treatments were used for gas sampling (Fig. S4b) and determination of N_2O emissions. Gas samples were also collected from the other four replicates initially, but sampling was stopped on the 11th day of incubation when destructive soil sampling was performed (Fig. S4c). The abundance of the fungi and fungal denitrifiers in the soil was estimated by quantitative PCR (qPCR) targeting a partial region of the 18S rRNA genes and *nirK* genes of fungi. The detailed qPCR methods are described in Supplementary Methods 3.

Statistical analysis

In the first field experiment, the differences in N_2O fluxes, cumulative N_2O emissions, and sweet corn yields between the groups were tested using an independent-samples t-test ($n = 6$, assuming equal variances, two tailed). In the second field experiment, the differences in cumulative N_2O emissions and mite abundances between the groups were tested by one-way ANOVA and LSD post hoc tests ($n = 3$). In the first soil microcosm experiment, the differences in cumulative N_2O emissions between the groups were tested by one-way ANOVA and Tukey's HSD post hoc tests ($n = 3$). The differences in mite abundances between the groups were tested using an independent-samples t-test ($n = 3$, assuming equal variances, two tailed). In the second soil microcosm experiment, the differences in cumulative N_2O emissions between the groups were tested by one-way ANOVA and Tukey's HSD post hoc tests ($n = 3$), and the differences in the abundances of fungal *nirK* genes and 18S rRNA genes in the soil between the groups were tested using independent-samples t-tests ($n = 4$, assuming equal variances, two tailed). These statistical analyses were performed using SPSS statistics 25.0 software (IBM Corp., USA). Linear regression between N_2O emissions and the mite abundance was performed using Microsoft Excel software (Microsoft Corp., USA).

Results

The application of coconut husks decreases N_2O emissions, and fungivorous mites are observed in the coconut husks

In the first field experiment, the N_2O emissions from all the plots started increasing 1 week after fertilizer application (Fig. 2c). The N_2O fluxes from the plots with coconut husk

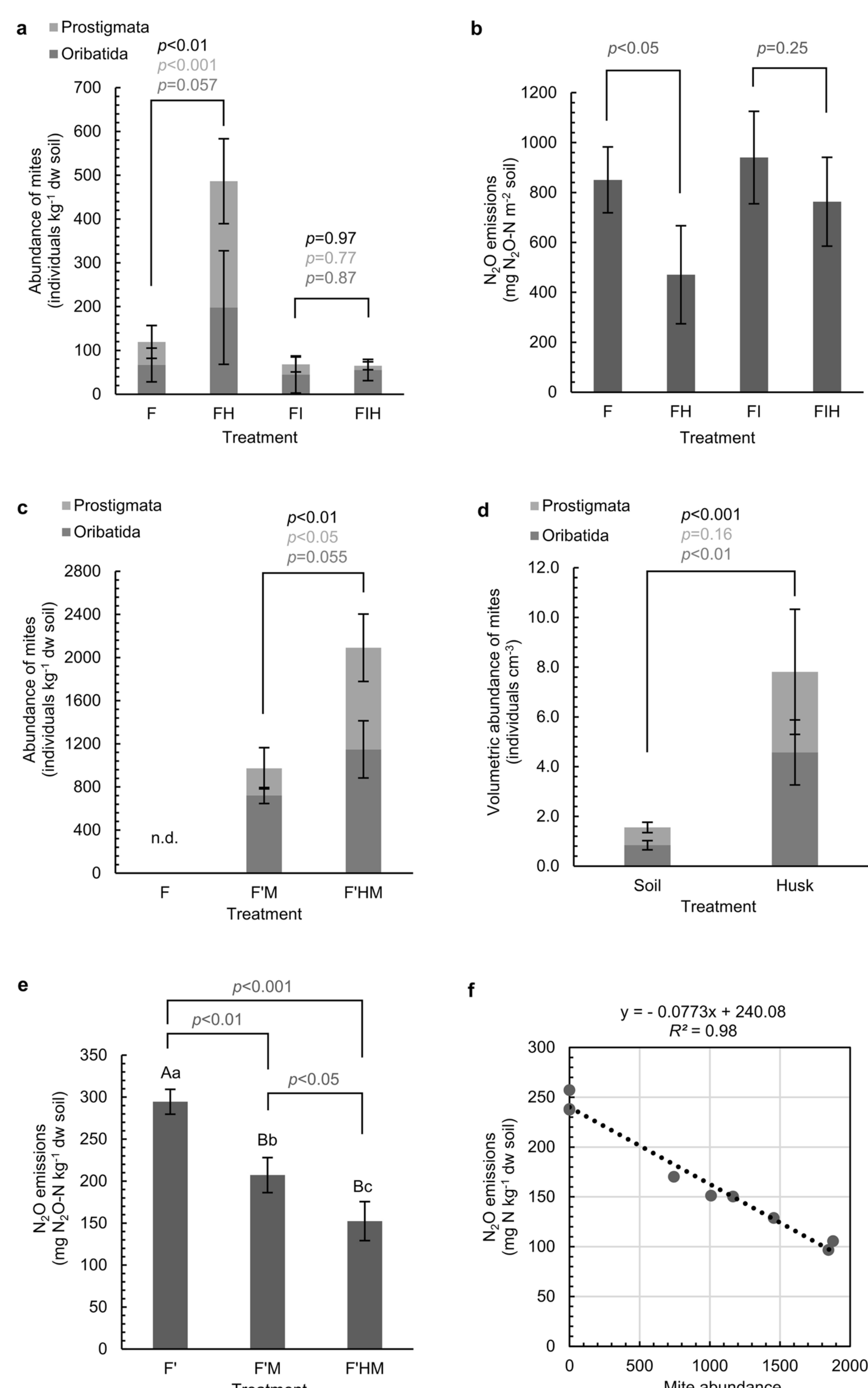
application and those without coconut husk application peaked on the same day (on May 29, June 29, and July 6), but the height of all the N_2O flux peaks from the plots with coconut husk application was lower than that from the plots without coconut husk application (Figs. 2c and S5). As a result, the cumulative N_2O emissions during the entire cultivation period from the plots with coconut husks were 53% lower than those from the plots without coconut husk application (Fig. 2d). Coconut husks did not impact the cultivated sweet corn because the mean value (± standard deviation, $n = 6$) of the sweet corn yields increased from 11.6 ± 1.6 to 11.9 ± 0.3 t/ha, although the difference was not statistically significant (Fig. 2e). Coconut husk application might even lead to a more stable yield among the plots because the interquartile range decreased from 3.1 to 0.6 t/ha (Fig. 2e). These findings suggest that the application of coconut husks to soil could mitigate N_2O emissions without impacting crop yields.

Using a stereomicroscope, many mites were observed in the gaps between the fibers of the coconut husks sampled from the field (Fig. 2f). The same mites were also found in the field soil without coconut husk application, but no mites were found in the newly unpacked coconut husks, which indicated that the mites living in the coconut husks sampled from the field immigrated from the field soils. The mites found in the coconut husks belonged to the two dominant mite species in the soil of the experimental field (Fig. 2g). The brown-colored species had a sclerotized exoskeleton, which is a typical morphological property of oribatid mites (suborder Oribatida) [22, 25, 40]. The other species, which were white to slightly yellow-green in color and had a soft body, are likely to be prostigmatic mites (suborder Prostigmata) according to the characteristic description provided by Krantz et al. [40]. The species were further identified based on DNA sequence information (Fig. 2g and Table S1). The first 472 amino acids of the sequenced region of the white mites were homologous (94%) to sequences from *Benoinyssus aff. serratus AD1053* (family: Eupodidae; Suborder: Prostigmata). The first 622 amino acids of the sequenced region of the brown mites were homologous (97%) to sequences from *Scheloribates pallidulus* (family: Scheloribatidae; Suborder: Oribatida). Oribatid mites are the most numerous microarthropods [22]. Information on the feeding habits of the mites was obtained based on their taxa. Most Oribatid mites feed on fungi, but these mites might also feed on decomposing organic matter [22]. In our study, after the mites were transferred to the soil surface, they immediately started grazing on fungal mycelia that had grown on the soil particles (Fig. 2h and Supplementary Video 1), which confirmed that these mites were fungivores. Prostigmatic mites include both predator species and microbial feeder species [22]. Species of the family Eupodidae are known to be fungivores and are commonly observed in plowed and fertilized agricultural fields [22].

Coconut husks decrease N_2O emissions by increasing the abundance of fungivorous mites in soil

In the second field experiment, both the Prostigmata and Oribatida populations increased after coconut husk application when miticide was not applied to the soil (similar conditions to those in the first experiment) (Fig. 3a). The abundances of Prostigmata and Oribatida in soils with coconut husk application (FH) were 449 and 196% higher than those in soils without coconut husk application (F), which corresponded to a 307% higher total mite abundance in the soils with coconut husk application. In contrast, when miticide was applied to the soil, no significant difference in the mite abundance was found between the soils with coconut husk application (FIH) and those without coconut husk application (FI), and the mite abundance in both soils was slightly lower than that found in soils without any treatment (F). The differences in the abundances of each mite species were also not significantly different. These results indicated that coconut husks increased the mite population, and this effect was removed when miticide was applied to the soil. The N_2O emissions decreased (Figs. 3b, S6, and S7) when the mite abundance increased, as was observed after coconut husk application in soils without miticide (Fig. 3a). The cumulative N_2O emissions from the soils with coconut husk application (FH) were 45% lower than those from soils without coconut husk application (Fig. 3b). In contrast, when soils were treated with miticide, which removed the increase in mite abundance observed with the application of coconut husks (Fig. 3a), the cumulative N_2O emissions from the soils with coconut husk application (FHI) were not significantly different from those from the soils without coconut husk application (FI) (Fig. 3b). These results indicated that the effect of coconut husks on reducing N_2O emissions relies on the activity of fungivorous mites.

In the first microcosm experiment, both the Prostigmata and Oribatida populations were increased by coconut husk application to soils containing mites (Fig. 3c), which was consistent with the results from the second field experiment (Fig. 3a). The abundances of Prostigmata and Oribatida in soils with coconut husk application (FH) were 59 and 274% higher than those in soils without coconut husk application (F), resulting in a 115% higher total mite abundance in soils with coconut husk application. Moreover, the abundances of mites in both the coconut husks and the soil were determined (for the F′HM treatment). The volumetric abundance of mites in the coconut husks was 5.02-fold higher than that in the soil, and for each mite suborder, Prostigmata and Oribatida, the corresponding values were 4.54-fold and 5.43-fold, respectively (Fig. 3d). These results were consistent with the finding from the first field



experiment that a large number of mites were observed in coconut husks recovered from the field (Fig. 2f). Mite application to mite-free soils (F′M) decreased N_2O emissions by 36% compared with the emissions from the control soils without mite application (F′) (Figs. 3e and S8). Moreover, the increase in the mite abundance after the addition of coconut husks (Fig. 3c) further decreased the N_2O emissions (F′HM) by 30% compared with the emissions from the soils with mite application but without coconut husks (F′M) (Figs. 3e and S8), which was consistent with the results from the second field experiment (Fig. 3a, b). A strong correlation ($R^2 = 0.9761$) was found

◀ **Fig. 3 Increased mite abundances in soils with coconut husk application and the resulting effect on N_2O emissions from soils.** Mite abundances (**a**) and cumulative N_2O emissions (**b**) in the second field experiment. The values represent the means ± SDs ($n = 3$). The p values were calculated by one-way analysis of variance (ANOVA) and LSD post hoc test and are indicated in the figures. The p values in black, gray, and orange correspond to the abundance of total mites, the abundance of Prostigmata, and the abundance of Oribatida, respectively. F: control, soils applied with fertilizer only; FH: soils applied with fertilizer and amended with coconut husks; FI: soils applied with fertilizer and miticide; FHI: soils applied with fertilizer and miticide and amended with coconut husks. **c** Mite abundances at the end of the incubation of the microcosms in the first microcosm experiment. The values represent the means ± SDs ($n = 3$). The p values were calculated by independent-sample t-tests (assuming equal variances, two tailed) and are indicated in the figures. The p values in black, gray, and orange correspond to the abundance of total mites, the abundance of Prostigmata, and the abundance of Oribatida, respectively. The term "n.d." indicates that the value was too low to be detected. F′: control, soils applied with fertilizer only; F′M: soils with fertilizer and mite applications; F′HM: soils with fertilizer, coconut husk, and mite applications. **d** Volumetric abundances of mites in the coconut husks and in the soil (treatment F′HM) in the first microcosm experiment. The values represent the means ± SDs ($n = 3$). The p values were calculated by independent-sample t-tests (assuming equal variances, two tailed) and are indicated in the figures. The p values in black, gray, and orange correspond to the abundances of total mites, the abundance of Prostigmata, and the abundance of Oribatida, respectively. Husk: coconut husks applied to the soil in the F'HM treatment; Soil: the soil with the F'HM treatment. **e** N_2O emissions in the first microcosm experiment. The values represent the means ± SDs ($n = 3$). The p values were calculated by one-way analysis of variance (ANOVA) and Tukey's HSD post hoc test and are indicated in the figures. F′: control, soils applied with fertilizer only; F′M: soils with fertilizer and mite applications; F′HM: soils with fertilizer, coconut husk, and mite applications. **f** Correlations between N_2O emissions and mite abundance. The green dots indicate the cumulative N_2O emissions (Y-axis) and the mite abundances in soils (X-axis, the contribution of mites extracted from the coconut husks was not included) at the end of the incubation of all the soil microcosms in the first microcosm experiment (color figure online).

between the mite abundance and N_2O emissions based on the data from all microcosm replications (Fig. 3f).

Fungivorous mites decrease N_2O emissions by consuming N_2O-producing fungi

In the in vitro test, the capability of mites to feed on N_2O-producing fungi was tested using pure cultures of N_2O-producing fungal strains belonging to a variety of taxa (the genera *Penicillium*, *Fusarium*, *Talaromyces*, *Trichoderma*, and *Aspergillus*). These strains were isolated from the experimental field and were verified to be able to produce N_2O under pure-culture conditions (Table S2). The results showed that the mites started grazing the mycelia immediately after being placed on agar plates cultured with each of the tested fungal strains (Figs. S9a–S9e and Supplementary Videos 2–6), which suggested that the mites exhibit wide-spectrum feeding behavior against N_2O-producing fungi. In particular, the results obtained with agar plates cultured with *Talaromyces* sp. revealed that each oribatid mite consumed approximately twofold more of its mycelial body size in 10 min (Fig. 4a), which indicated that the consumption of N_2O-producing fungi by mites could be very rapid.

In the second microcosm experiment, the capability of the mites to feed on N_2O-producing fungi in soil was estimated. On day 10 after fertilization, the abundance of the fungal *nirK* gene, which was used as the marker gene of N_2O-producing fungi, in soils applied with mites was 21% lower than that in mite-free soils (Fig. 4b), which indicated that mites decreased the abundance of N_2O-producing fungi. Moreover, the abundance of fungal 18S rRNA genes was 42% lower (Fig. 4c), which indicated that mites also decreased the abundance of total fungal communities. The rapid shift in the abundance of fungal denitrifiers in soil after mite introduction was consistent with their rapid consumption of mycelia of fungal denitrifiers in vitro (Fig. 4a). N_2O emissions decreased (Fig. 4d, e) as the abundance of N_2O-producing fungi decreased (Fig. 4b), which occurred after mite application to the soils. The N_2O emissions from all the microcosms peaked on the same day, which indicated that neither the application of mites nor the application of coconut husks to the soils changed the N_2O emissions pattern (Fig. 4d). However, the height of the N_2O emission peak in the mite-applied soils (F′M) was distinctly lower than that in the mite-free soils (F′) (Fig. 4d), which corresponded to 35% lower cumulative N_2O emissions from the mite-applied soils (Fig. 4e). In contrast, the N_2O fluxes from soils applied with coconut husks (without mites, F′H) were almost the same as those from the untreated control soils (F′) throughout the N_2O emission period after fertilization (Fig. 4d), which resulted in cumulative N_2O emissions that were not significantly different (Fig. 4e). These results indicated that coconut husks could not affect N_2O emissions in the absence of mite activities, which was consistent with the results from the second field experiment. The N_2O emission peak obtained for the mite-applied soils (F′M) from the microcosm replicates used for destructive soil sampling was also lower. In particular, 10 days after fertilization, when the gene abundances were determined, the N_2O fluxes from the soils applied with mites (F′M) were 29% lower than those from the mite-free soils (F′, Fig. S10a). The cumulative N_2O emissions from these replicates of mite-applied soils (F′M) were 32% lower than those from mite-free soils (F′, Fig. S10b), which were similar to the results from other replicates shown in Fig. 4e.

Discussion

Coconut husks applied to soil provide a preferred habitat for mites that facilitates their reproduction and increases their abundance in soil (hypothetical step 1 shown in Fig. 1), as was observed in the second field experiment and the first

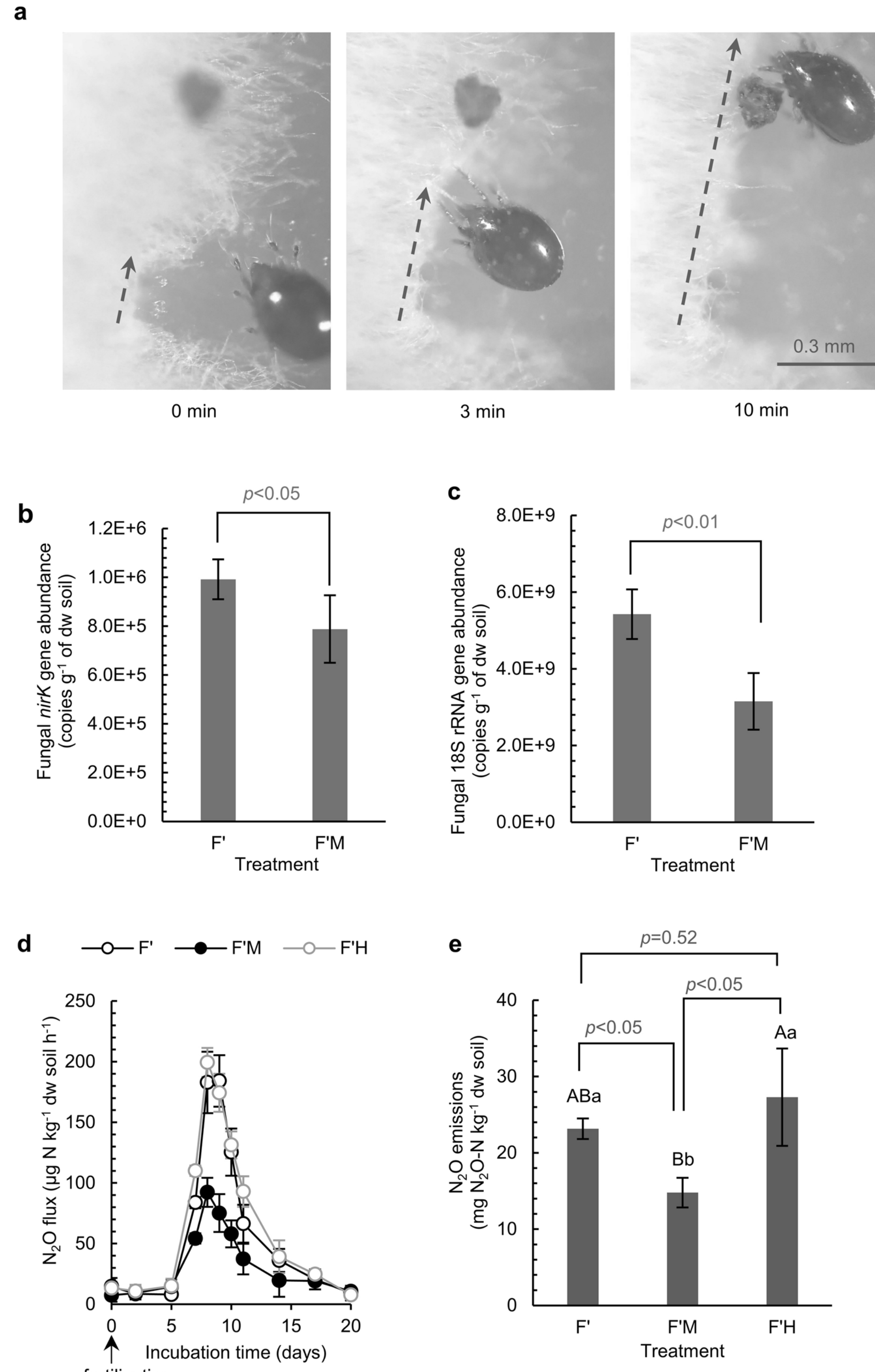


Fig. 4 Consumption of N_2O-producing fungi by fungivorous mites and the subsequent effect on N_2O emissions from soils. a Consumption of *Talaromyces* sp. grown on agar plates by fungivorous mites. The numbers below the pictures represent the times at which the photographs were captured. The arrows indicate the sites at which the mites fed on mycelia. The bar indicates 0.3 mm. The brown object next to the mite is a small soil particle dropped on the agar plate when transferring the mite to the plate. The soil particles did not affect the observations over the short term. Abundances of fungal *nirK* genes (**b**) and 18S rRNA genes (**c**) in soil in the second microcosm experiment. The values represent the means ± SDs ($n = 4$). The p values were calculated by independent-sample t-tests (assuming equal variances, two tailed) and are indicated in the figures. F′: control, soils applied with fertilizer only; F′M: soils applied with fertilizer and amended with mites. N_2O fluxes (**d**) and cumulative N_2O emissions (**e**) in the second microcosm experiment. The values represent the means ± SDs ($n = 3$). The p values were calculated by one-way analysis of variance (ANOVA) and Tukey's HSD post hoc test and are indicated in the figures. F′: control, soils applied with fertilizer only; F′M: soils applied with fertilizer and amended with mites; F′H: soils applied with fertilizer and amended with coconut husks. The arrow indicates the date of fertilization (color figure online).

microcosm experiment. Coconut husk application clearly increased the dominant mite populations, Prostigmata and Oribatida, in both the field experiment (Fig. 3a) and the microcosm experiment (Fig. 3c). The increase in the abundance of Prostigmata appeared to be greater than that in the abundance of Oribatida (Fig. 3a, c). This finding might have been obtained because some groups of Prostigmata, such as the family Eupodidae, are opportunists that are able to reproduce rapidly following a disturbance or a sudden shift in resources, whereas Oribatida are known to reproduce relatively slowly relative to other micro-arthropods [22]. The coconut husks could promote mite reproduction by giving mites a favorable habitat because large numbers of mites were observed to be living in

the gaps between the fibers of the coconut husks recovered from the field (Fig. 2f, g) and the volumetric abundance of mites in the coconut husks was markedly higher than that in the soil in the microcosm experiment (Fig. 3d). The preference of mites to live in coconut husks could be due to the porous structure of the husks, which makes them highly permeable to air and water [41]. This type of environment could be favorable for the habitation and oviposition of mites [21, 24–26]. Notably, the minute structures of coconut husks are very similar to leaf domatia (also called mite domatia), which are morphogenetic structures on the undersides of the leaves of some plant species and are usually occupied by mites [42]. Mite domatia provide mites and their eggs with shelter from desiccation [42, 43] and a refuge from predation [42, 44]. More interestingly, it has been reported that clones and cultivars of grape with larger leaf domatia support more fungivorous mites and suffer less fungal damage [42, 45], which indicates a potentially similar ecological basis for the mitigation of N_2O observed with coconut husks in our study. In addition to increasing the mite abundance, coconut husk application decreased N_2O emissions in both the field experiment (Fig. 3b) and the microcosm experiment (Fig. 3e), whereas coconut husks did not affect N_2O emissions in soils where mite activities were suppressed by miticide in the field experiment (Fig. 3b, e) or in mite-free soils in the microcosm experiment (see the second microcosm experiment, Fig. 4e). These results confirmed that the effect of the application of coconut husks on N_2O emissions relies on mite activities, which suggests that hypothetical steps 2 and 3 are necessary.

The fungal feeding activities of mites might decrease the abundance of N_2O-producing fungi by consuming their biomass in soil (hypothetical step 2 shown in Fig. 1), as was observed in the in vitro tests of the feeding behaviors of mites with N_2O-producing fungal strains and the second soil microcosm experiment. The analysis of the feeding habits showed that mites can rapidly feed on various isolated N_2O-producing fungal strains (Figs. 4a and S9a–e and Supplementary Videos 2–6), and the microcosm study showed that the addition of mites to mite-free soils significantly decreased the abundance of denitrifying fungi (Fig. 4b) as well as the total fungal abundance (Fig. 4c). Feeding is the most likely method through which mites affect the fungal abundance in the tested soils. Although mites are also capable of affecting the fungal abundance via non-consumptive interactions, such as spore dispersal [46] and habitat formation [21], these effects generally take a long time [21]. In contrast, the fungal abundance in the tested soils decreased within a relatively short period after the introduction of mites to the tested soils. Specifically, 11th day after mite introduction, the fungal abundance in the mite-applied soils was half of that in the mite-free soils (Fig. 4b), and the analysis of the N_2O fluxes, which showed that the fluxes from the soils with mites on the 5th day was 50% of those from the mite-free soils (Fig. 4d), indicates that the difference in fungal abundance probably appeared even earlier. In addition, the likely general fungal feeding habits observed in the in vitro test, which agreed with the reports that many oribatid mites appear to be indiscriminant fungal feeders [22], could explain why mite addition not only decreased the abundance of denitrifying fungi but also that of the total fungal abundance in the microcosm study.

The decreases in the abundance of fungal denitrifiers and the total fungal abundance observed after mite addition were accompanied by decreased N_2O emissions (Fig. 4b–e), which suggests that mites could decrease N_2O emissions by decreasing the abundance of N_2O-producing fungi in soil (hypothetical step 3 shown in Fig. 1). Interestingly, the rate of decrease in N_2O emissions was 85% of that found for fungal abundance at the N_2O flux peak (Fig. 4c, e). These results agreed extremely well with the findings obtained in our previous study, which suggest that fungi contribute up to 84% of N_2O emissions under similar conditions (using the same fertilizer and soil type as those used in this study) [15]. Therefore, the decreased production of N_2O by fungal denitrifiers might be the essential reason for the lower emissions of N_2O after the addition of mites to the tested soils. However, it should be noted that the direct production of N_2O is likely not be the only way that fungi influence N_2O dynamics. Saprotrophic fungi play an important role in nitrogen mineralization by decomposing organic matter [33], and through their effect on nitrogen availability, these fungi could indirectly influence bacterial nitrification and denitrification [21]. Thus, the decrease in fungal abundance might reduce the N_2O derived from bacterial nitrification and denitrification. In contrast, although fungivorous mites might decrease mineralization rates by decreasing the fungal abundance, most oribatid mites are also thought to be capable of facilitating mineralization processes by fragmenting organic matter while feeding on fungi adhering to detritus [22, 47]. A recent study showed that the addition of mesofauna (combination of enchytraeids, fungivorous mites, and predatory mites) to soil increases N_2O emissions by enhancing nitrogen mineralization [29]. Therefore, whether and to what extent the mites in our system affect N_2O emissions by affecting nitrogen mineralization remain to be studied.

In summary, our results uncovered that mite feeding exert a negative regulatory effect on N_2O emissions and demonstrated that this regulation could be enhanced by modifying the soil habitat of mites and could thus be used for the mitigation of N_2O emissions. Before our study, although it is well known that fungivorous mites are widespread in agricultural soils and that their fungal feeding behaviors comprise an integral section of the food web of agroecosystems [21, 24], whether fungal consumption by

mites is sufficiently intensive to affect the abundance of fungi, which usually relies on the relative population of mites to fungi, remains unclear [21]. Our results showed that a mite abundance in the range of from hundreds (in the second field experiment and second microcosm experiment) to a few thousands (in the first microcosm experiment) per kg of soil could be sufficient to significantly affect the fungal abundance (Fig. 4b, c) and N_2O emissions (Fig. 4e). With a mite abundance in the range of ~0–2000 mites per kg of soil (first microcosm experiment), the N_2O emissions correlated extremely well with the mite abundance (Fig. 3f), which suggests that the regulation of fungal feeding by mites could be very strong when N_2O emissions are dominated by fungal denitrification, such as after the application of an organic fertilizer to the soil. The finding of the regulatory effect of the microbial feeding behaviors of mesofauna on N_2O production provides an important advancement to our understanding of N_2O emissions from soils. Most studies on the mitigation of N_2O emissions have focused on microbiota because these contain producers of N_2O, whereas mesofauna have rarely been linked to N_2O emissions. Our findings demonstrated that in addition to microbiota, mesofauna, as predators of N_2O producers, should also be an integral part of studies on the mechanisms and sources/sinks of N_2O emissions.

Through the use of coconut husks, the fungivorous mite population in soil could be increased at the field scale, and these findings provide a method for mitigating N_2O emissions from agricultural soils. This strategy involves the application of natural materials to soil and the use of the microbial feeding behaviors of soil mesofauna that exist naturally in the soil food web rather than the application of synthesized compounds, and thus, the proposed strategy might be more environmentally friendly than conventional chemical-based methods [8, 48]. Nevertheless, it should be noted that the method was only tested using a specific type of organic fertilizer and a specific type of soil (Andisol) in the present study; thus, the universality of the method needs to be tested using different types of fertilizer and soil. In addition, the mitigation of N_2O emissions through the use of the fungal feeding behaviors of mites observed in this study could be based on the premise that fungal denitrification is the main N_2O production process in agricultural soils after organic fertilizer application, which suggests that the efficacy of this approach in soils where bacterial nitrification and denitrification are the main N_2O production processes might need to be further researched.

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Author contributions HS, YS, and KS conceived of the study. YS designed the first field experiment and interpreted the raw data. YS and SO performed the field setup, gas sampling, and corn yield determination in the first field experiment. HS extracted the mites from coconut husks and identified the mites. HS, KS, and YS proposed the hypothesis. HS, YS, and KS designed the second field experiment. YS, HS, KS, and SO performed the field setup, gas sampling, soil sampling, and mite extraction in the second field experiment. HS counted and identified the mites in the second field experiment. HS designed and performed all in vitro experiments and soil microcosm experiments. YM supported DNA extraction, qPCR, and the relative data analysis. HS performed the statistical analyses. HS wrote the paper with contributions from KI and KS.

Competing interests The authors declare no competing interests.

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