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3Nitrogen addition impacts **on the emissions of greenhouse gases** depending on the forest
4type: a case study in Changbai Mountain, Northeast China

5

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21

22Abstract

23*Purpose* Anthropogenic-induced greenhouse gas (GHG) emission rates derived from the soil are
24influenced by long-term nitrogen (N) deposition and N fertilization. However, our understanding
25of the interplay between increased N load and GHG emissions among soil aggregates is
26incomplete.

27*Materials and methods* Here, we **conducted** an incubation experiment to explore the effects of soil
28aggregate size and N addition on GHG emissions. The soil **aggregate** samples (0-10 cm) were
29collected from two six-year N addition experiment sites with different vegetation types (mixed
30Korean pine forest vs. broad-leaved forest) in Northeast China. Carbon dioxide (CO₂), nitrous
31oxide (N₂O), and methane (CH₄) production was quantified from the soil samples in the laboratory
32using gas chromatography **with 24h**-intervals during the incubation (at 20°C for 168 h with 80%
33field water capacity).

34*Results and discussion* The results showed that the GHG emission/uptake rates were significantly
35higher in the micro-aggregates than in the macro-aggregates **due to the higher concentration of soil**
36**bio-chemical properties (DOC, MBC, NO₃⁻, NH₄⁺, SOC and TN) in smaller aggregates.** For the
37**N addition treatments, the emission/uptake rates of GHG decreased after N addition across**
38**aggregate sizes especially in the mixed Korean pine forest (PF) where CO₂ emission was**
39**decreased about 30%. The significant effects of N addition on GHG emissions on SOM basis were**
40**recorded in the PF rather than in the broad-leaved forest (SF) suggested that N addition decreased**
41**of GHG emission/uptake rates through change in the quality of SOM in the PF forest. The global**
42**warming potential (GWP) which was mainly contributed by CO₂ emission (>98%) was decreased**
43**in the PF after N addition but no changes in the SF.**

44*Conclusions* These findings suggest that soil aggregate **size** is an important factor controlling
45GHG emissions through mediating the content of substrate resources in temperate forest
46ecosystems. The inhibitory effect of N addition on the GHG emission/uptake rates depends on the
47forest type.

48

49**Keywords** Greenhouse gas • N deposition • Soil aggregate • Soil organic carbon

501 Introduction

51 Anthropogenic-induced greenhouse gas (GHG) emissions produced from soil to the
52 atmosphere have increased steadily since the industrial revolution . Carbon dioxide (CO₂),
53 nitrous oxide (N₂O) and methane (CH₄) are the main components of GHGs, which accounts
54 for more than 80% of global warming . The increasing concentrations of GHGs are set to
55 continue in many industrialized regions, including the eastern regions of China . Of the total
56 anthropogenic emissions (8.6 Gt C yr⁻¹), only half are absorbed by the ocean or by
57 unidentified terrestrial sinks . The mitigation of GHG emissions from the terrestrial ecosystem
58 requires the reduction of direct emissions from sources through the enrichment of **carbon (C)**
59 **and nitrogen (N)** sinks in the soil . Temperate forest ecosystems sustain approximately 20% of
60 the world's plant biomass and 10% of terrestrial C. In addition, temperate forests have the
61 potential to store and sequester C and therefore, mitigate global warming; however, they can
62 be influenced by various factors, including biotic (e.g. anthropogenic activity) and abiotic
63 (e.g. N deposition) factors .

64 The increased concentration of GHGs can in turn impact the decomposition of soil organic
65 matter (SOM) and carbon storage. The increased concentration of N₂O in the atmosphere has
66 doubled the rate of N inputs into most terrestrial ecosystems . The effect of N deposition on
67 SOM decomposition and C storage has received significant attention in recent years. For
68 example, the increased N deposition due to the increased concentration of N₂O in the
69 atmosphere has been found to increase , decrease or have no effect on the soil C content. In
70 addition, the increased N deposition is reported to promote soil GHG emissions/uptakes due
71 to changes in soil N status and microbial communities . This effect could increase GHGs in
72 the atmosphere and subsequently aggravate global warming. However, there is still a
73 significant knowledge gap in our understanding of the mechanisms by which N deposition
74 directly impacts GHG releases and C storage through changes in the chemical and biological
75 properties of soil . Studies on the effects of soil structure or soil aggregate distribution on
76 GHG emissions due to N deposition are rare; however, these studies are urgently needed to
77 facilitate decision-making in forest management. [Diba et al. \(2011\)](#) reported that N₂O

78emissions could be significantly influenced by soil aggregate size and N addition due to
79differences in soil properties in various aggregate sizes.

80 The physical protection of SOM through the formation of organo-mineral associations
81within soil aggregates is one of the most crucial but poorly understood stabilization
82mechanisms of SOM in soil . Soil aggregates physically protect the SOM from various
83decomposing agents and then stabilize the SOM through several mechanisms, including
84through separated enzymes and substrates on mineral and humic surfaces , suppressed
85microbial activity due to inhibited access to gases or nutrients , and separated decomposer
86organisms to SOM within small porous systems . These mechanisms suggest that the
87locations of SOM and the soil nutrient properties in aggregates control the rate of SOM
88decomposition and therefore, control the emission rates of GHGs. The emission rates of
89GHGs from SOM decomposition differ significantly among aggregate sizes (e.g. macro-
90aggregates vs. micro-aggregates; [Six and Paustian \(2014\)](#)). [Tao and Song \(2013\)](#) found that C
91mineralization was higher in micro-aggregates than in macro-aggregates in clay loam soil.
92However, [Elliott et al. \(1996\)](#) observed a contrasting trend and found that the rate of C and N
93mineralization was greater in macro-aggregates than in micro-aggregates. [Mangalassery et al.](#)
94[\(2013\)](#) reported that the CO₂, N₂O and CH₄ emission/uptake rates were greater in the micro-
95aggregates than in the macro-aggregates with clay loam texture; however, the emission/uptake
96rates were greater in macro-aggregates than in micro-aggregates with sandy loam texture.
97Therefore, although SOM in macro-aggregates was more labile and less processed than that in
98micro-aggregates, the concentration of SOM in micro-aggregates under clay-enriched soil
99was much higher than in macro-aggregates and subsequently resulted in higher emission rates
100of GHGs. However, [Aoyama et al. \(1999\)](#) reported that crushing macro-aggregates enhanced
101C mineralization by 14-35%. Similar observations were also made by [Mueller et al. \(2012\)](#),
102who reported that the dispersion of aggregates using ultrasonic treatment resulted in an
103increase in C mineralization by 27% due to an increase in labile organic carbon. Therefore,
104the emission rates of GHGs in soil aggregates can be controlled not only by soil properties but
105also by aggregate stability.

106 The majority of recent studies have focused on the effects of land use and management
107 on soil aggregation and GHG emissions, but few studies have determined the effect of N
108 deposition on soil aggregation and the emission rates of GHGs in aggregates. However, [Plaza-](#)
109 [Bonilla et al. \(2014\)](#) observed that inorganic N fertilization promotes the emission of CO₂ and
110 N₂O in an incubation experiment using agricultural soil. [Diba et al. \(2011\)](#) detected that the
111 impact of N addition on N₂O emission depended on soil aggregate size in grassland soil. [Tao](#)
112 [and Song \(2013\)](#) reported that N addition had a neutral and negative effect on SOC
113 decomposition in wetland soil. It is generally believed that the patterns of GHG emissions are
114 less evaluated in soil aggregates in forest ecosystems that incur significant N deposition,
115 especially in Northeast China (> 23 kg N yr⁻¹ ha⁻¹).

116 Therefore, a six-year *in situ* N addition experiment in mixed Korean pine forest and
117 broad-leaved forest was established at Changbai Mountain in Jilin Province, northeastern
118 China. We hypothesized the following: (1) N addition would induce positive effects on GHG
119 emissions in the temperate forest soil; (2) compared with the mixed Korean pine forest, the
120 broad-leaved forest would provide resources richer in nutrients, which would translate into
121 increased GHG emissions, and (3) the small-sized aggregates with a larger surface area
122 would capture more nutrients than the larger aggregates and therefore, would produce higher
123 GHG emissions. To test these hypotheses, first, the concentration of C and N, and other
124 properties of the aggregates were determined from two N addition experiment sites with
125 different vegetation composition. Second, the GHG emission rates from the soil aggregates
126 were measured in a laboratory incubation experiment.

127

1282 Materials and methods

1292.1 Site location

130 The study sites were located in the research forest of the Changbai Forest Ecosystem
131 Research Station (CBFERS; established in 1979) in Northeast China. A secondary forest (SF)
132 and a primary forest (PF) were selected for the study. The SF is a 30-year-old broad-leaved
133 forest (42.517° N, 127.783° E, Jilin Province, China) and is dominated by birch (*Betula*

134 *platyphylla* Suk) and aspen (*Populus davidiana*) trees. The mean annual precipitation and
135 temperature at this site are 871.6 mm and 2.7°C, respectively. The PF is a mixed Korean pine
136 old growth forest and is close to the city of Erdaobaihe (42.402N, 128.096E). The mean
137 annual precipitation and temperature at this site are 700mm and 3.8 °C. The dominant plant
138 species in the PF are *Quercus mongolica* and *Pinus koraiensis*. The soil at both study sites is
139 classified as Mollisols after the American Soil Taxonomy with high organic matter content in
140 the surface soil layer. The ambient N deposition, measured from throughfall at the study sites,
141 is 23 kg N ha⁻¹ yr⁻¹. A more detailed description of the study sites is presented in Table 1.

142

143 2.2 Experimental design

144 Two levels of N addition (0 kg N ha⁻¹ yr⁻¹ (N0); 50 kg N ha⁻¹ yr⁻¹ (N50)) with three replications
145 were randomly arranged into 6 blocks at both sites (30×30 m for each block in SF and 25×50
146 m for PF). Ammonia nitrate (NH₄NO₃) aqueous solution (40 L) was applied to the treated
147 plots monthly during the growing season (4-10 months) each year starting in 2007 (SF) and
148 2009 (PF). Four soil samples from each block were collected to make one composite sample
149 from the A-horizon (0-10 cm) in October 2012 (SF) and May 2014 (PF). The soil samples
150 were brought into the laboratory where plant litter, fine roots, and larger soil fauna were
151 removed. The remaining soil was then gently sieved using an 8-mm sieve and air-dried at
152 room temperature. [The soil was then separated into five sizes of aggregates and stored at 4°C](#)
153 [before incubation.](#)

154

155 2.3 Aggregate preparation

156 [The air-dried soil samples were passed through a series of sieves \(4 mm, 2 mm, 1 mm, 0.25](#)
157 [mm and 0.053 mm\) using the wet sieve method to obtain five aggregate size-fractions of 4-8](#)
158 [mm, 2-4 mm, 1-2 mm, 0.25-1 mm, and 0.053-0.25 mm \(free micro-aggregates, free-m\).](#)
159 Briefly, the air-dried soil (100 g) was moistened in distilled water for 10 min and then shaken
160 in a container with distilled water for 10 min. The separated aggregates were oven-dried at
161 60°C, and then weighed. The field water capacity (FWC) was measured using a funnel

162 according to the methods of [Diba et al. \(2011\)](#), and the values of the FWC from the largest
163 aggregates to the smallest aggregates were 1.01 g g⁻¹, 1.03 g g⁻¹, 1.18 g g⁻¹, 1.59 g g⁻¹, 1.99 g g⁻¹,
164¹, respectively.

165

166 2.4 Incubation procedure

167 The soil aggregate samples (10 g each, dry) were loosely packed into 300 ml incubation
168 flasks. All samples for each aggregate size (with 3 empty flasks as controls) were adjusted to
169 80% FWC moisture and then balanced for 2 h before gas sampling. After the first sampling,
170 all samples were incubated at 20°C for 168 h. The flask necks were sealed by a semi-
171 permeable membrane to prevent the samples from drying. The gas samples from the dry soil
172 (before incubation) and wet soil (after soil wetting) were taken using a 100 ml syringe and
173 then analyzed by gas chromatography (Agilent 7890A, USA). The concentrations of CO₂ and
174 CH₄ were detected using an infrared ion detector and the concentration of N₂O was detected
175 using an electron capture detector. The gas samples were taken at regular intervals (2 h, 24 h,
176 48 h, 72 h, 120 h, and 168 h). The levels of NH₄⁺-N, NO₃⁻-N, dissolved organic carbon
177 (DOC), and microbial biomass carbon (MBC) of the aggregate soils were measured before
178 and after incubation, and the total carbon (TC) and total nitrogen (TN) levels were measured
179 before incubation.

180 We performed sand-corrections to analyze the concentration of C and N in the aggregate
181 fractions using the following formula (Eq. (1)):

$$182 \text{ Sand free } C_{fraction} = \frac{C_{fraction}}{1 - sand_{fraction}} \quad (1)$$

183 where $C_{fraction}$ is the C concentration (g kg⁻¹) in the different aggregate sizes and $sand_{fraction}$ is the
184 fraction of sand in that aggregate size. To calculate the sand free N in the aggregate fraction,
185 the concentration of N in the fraction was used instead of the C concentration in Eq. (1).

186 The gas production rate (F, mg kg⁻¹ h⁻¹ or µg kg⁻¹ h⁻¹) was calculated according to the
187 methods of [Diba et al. \(2011\)](#) using the following equation (Eq. (2)):

$$188F = \rho \times \frac{V}{W} \times \frac{\Delta c}{\Delta t} \times \frac{273}{T} \quad (2)$$

189where F is the gas emission rate (mg kg⁻¹ Agg h⁻¹) of the aggregates, ρ is the density of gas (μg
190m⁻³) at 273 K and 1 atm., V is the headspace volume of the flask (m³), W is the dry soil weight
191(kg), Δc is the gas concentration change in the flask (m³ m⁻³), Δt is the incubation period (h),
192and T is the air temperature (K) in the flask.

193 The cumulative gas emission during the incubation was calculated using the following
194equation (Eq. (3)):

$$195\text{Cumulative F} = \sum(F_i \times T_i) \quad (3)$$

196where F_i is the mean gas production rate of the sampling intervals (mg kg⁻¹ h⁻¹) and T_i is
197number of hours in the sampling intervals.

198

1992.5 Contribution of SOM to GHG emissions from aggregate fractions

200 To determine the interaction effect of SOM and N addition on GHG emissions, the GHG
201emissions from each aggregate fraction were calculated on per SOM basis using the following
202equation (Eq. (4)):

203The GHG emission rate from SOM (mg kg⁻¹ SOM h⁻¹) = F (mg kg⁻¹ Agg h⁻¹) / SOM
204concentration in the aggregates (g kg⁻¹ Agg) × 10³ (4)

205

2062.6 Estimation of global warming potential

207The net global warming potential (GWP, mg CO_{2e} GHG emission kg⁻¹ Agg) was estimated
208using the radiative force in a time horizon of 100 years of the different greenhouse gas
209molecules . For computing the GWP, the GHG emission rate was expressed as a CO₂
210equivalent (CO_{2e}) using Eq. (5):

$$211\text{GWP} = \text{CO}_2(\text{g kg}^{-1} \text{ Agg}) + 298 \times \text{N}_2\text{O}(\text{g kg}^{-1} \text{ Agg}) + 25 \times \text{CH}_4(\text{g kg}^{-1} \text{ Agg}) \quad (5)$$

212where CO₂, N₂O and CH₄ represent the 7-day cumulative emissions of the individual GHGs.

213

2142.7 Statistical analysis

215 Statistical analysis was performed using SPSS 13.0 (SPSS Inc., USA). The effects of the two
216 factors (aggregate size (S) and N addition (N)) and their interactions on gas production and
217 the soil properties in each forest site were analyzed using a general linear model ANOVA.
218 Natural-log transformations were applied when it is necessary to meet the assumptions of
219 ANOVA. To determine the effects of soil initial properties on GHG emissions, the correlation
220 between GHG emissions and soil properties before incubation was analyzed using a two-
221 tailed Pearson's correlation at a significance level of 0.05.

222

2233 Results

2243.1 Soil properties in aggregates

225 The bio-chemical properties (NH_4^+ , NO_3^- , DOC and MBC) of the soil aggregates were
226 measured before and after incubation, and SOC and TN were measured before incubation
227 (Table 2). After incubation, the levels of NH_4^+ and NO_3^- increased in the SF, while the
228 opposite trend was observed in the PF. In addition, DOC increased and MBC decreased after
229 incubation at both the SF and the PF. In the aggregate size classes, all bio-chemical properties
230 were higher in the free-m, and lower in the macro-aggregates at both forest types. The
231 influence of N addition on the aggregate bio-chemical properties was not significant with the
232 exception of DOC before incubation at both forest sites. The concentration of DOC in
233 aggregates decreased after N addition in the SF, but increased in the PF.

234

2353.2 Aggregate gas release

236 At both forest sites, 7-day cumulative CO_2 emissions increased substantially during the first
237 seven days after the start of incubation. The CO_2 emissions in the PF were significantly
238 affected by aggregate size and N addition ($P < 0.01$, Table 3), while only affected by aggregate
239 size significantly in the SF. Cumulative CO_2 emissions increased as the aggregate size
240 decreased at both forest sites. At both forest sites, cumulative CO_2 emissions from the soil
241 aggregates were reduced after N addition, especially in the PF soil where CO_2 production was
242 reduced by approximately 30% compared with N_0 .

243 The 7-days cumulative N₂O emissions were significant affected only by aggregate size
244($P<0.01$, Table 3). N₂O emissions increased rapidly during the first 24 h after the start of
245incubation (Figs. 1 and 2). On average, the 7-days cumulative N₂O emissions under fertilized
246plots (9.36 $\mu\text{g kg}^{-1}$ Agg) were similar to control plots (8.97 $\mu\text{g kg}^{-1}$ Agg) in the SF, while
247slightly decreased (7.26 vs. 5.31 $\mu\text{g kg}^{-1}$ Agg for N0 vs. N50) after N addition in the PF across
248aggregate sizes. The 7-day cumulative N₂O emissions were lower in the medium-sized
249aggregates (2-0.25 mm) compared with the 4-8 mm and free-m aggregates for the N addition
250treatments at both forest sites.

251 Regarding the aggregate size, the cumulative CH₄ uptake was higher in the free-m
252aggregates compared with that in the macro-aggregates at both forest sites. Furthermore, the
253cumulative CH₄ uptake remained unchanged in the macro-aggregates in the PF, but increased
254as the macro-aggregate size decreased in the SF. The treatments of N addition showed no
255effect on the CH₄ uptake across all aggregate sizes ($P>0.05$, Table 3), although decreased CH₄
256uptake in the PF (~20%) and increased CH₄ uptake in the SF (~15%) were observed after N
257addition.

258

2593.3 Gas fluxes expressed in terms of SOM

260When the GHG flux data were expressed in terms of organic matter, the differences in all
261three gas flux patterns between aggregate sizes and N treatments were similar to the per soil
262patterns (Table 4). Briefly, higher CO₂ emission rates were recorded in the smaller aggregate
263sizes across the N addition groups. Compared with control, the emission rates of CO₂ were
264lower under fertilized samples at both forest sites. The patterns of the average CH₄ uptake
265rates expressed per SOM were higher in smaller aggregates in the SF site, while lower CH₄
266uptake rates were recorded at medium-size aggregates (0.25-2 mm) in the PF. The average
267CH₄ uptake rates were inhibited after N addition across aggregate sizes.

268 In all treatments, the average N₂O emission rates per SOM were similar to the average
269N₂O emission per soil across aggregate sizes and N addition (Table 4). However, the average
270N₂O emission rates per SOM after N addition treatment were significantly lower than the

271 control plots, especially in the PF soil ($P=0.024$).

272

273 3.4 Global warming potential

274 The CO₂ emissions were the greatest contributor to the GWP (Fig. 3). It was identified that
275 CH₄ contributed negatively to the GWP at both forest sites because of the net CH₄ uptake by
276 the soil during incubation. The GWP from the SF was significantly higher (148.3%) than that
277 of the PF soil. For N treatment, there was a significant difference in the GWP between N0 and
278 N50 in the PF, but no significant effect was found in the SF. An increase trend of GWP was
279 observed at both sites as the aggregate size decreased.

280

281 3.5 Relationships between gas emissions and soil properties

282 Pearson's correlation coefficients among GHG emissions and soil properties of before
283 incubation in each forest site were showed in Table 5. The relationships between soil initial
284 properties and GHG emissions differed between forest sites. In the SF, CO₂ emissions were
285 significant correlated to most of soil properties except for NO₃⁻ ($r=0.180$, $P>0.05$), while in
286 the PF only MBC, SOC and TN were significant correlated with CO₂ emissions ($r=0.440$,
287 0.502 , 0.550 respectively). The significant relationships between N₂O emissions and soil
288 properties were found only between DOC and N₂O emissions ($r=0.445$), and NO₃⁻ ($r=0.479$)
289 and N₂O emissions in the PF, but all of the soil properties were significantly correlated with
290 N₂O emissions despite of the MBC which was marginally significant ($r=0.323$, $P=0.087$). In
291 the PF, only MBC was marginally significant correlated with CH₄ uptake ($r=0.327$, $P=0.077$),
292 but in the SF, only MBC and TN did not have any relationship with CH₄ uptake. These
293 relationships differed between forest types indicated that soil nutrients in the SF were easier
294 for microbial utilization than in the PF.

295

296 Discussion

297 The objective of this study was to investigate the effects of soil aggregate and N addition on
298 GHG emissions in two different forest sites. Many factors, including the soil biotic and

299 abiotic properties of different sized aggregates, independently and synergistically influence
300 the emissions of GHG . The differences in soil properties observed before and after incubation
301 were statistically significant (Table 2). The decreased content of MBC after incubation may
302 be the main reason for the decrease in GHG emissions. The increased content of DOC may
303 result from the decreased stability of aggregates from the reciprocated force. The increased
304 content of NH_4^+ and NO_3^- after incubation is similar to results reported by [Diba et al. \(2011\)](#)
305 and can be attributed to N mineralization during incubation. In the present study, we found
306 that N addition and aggregate size influenced GHG emissions with the evidences of changes
307 in various soil properties (Table 2). The details were discussed below.

308

309 4.1 CO₂ emission

310 The CO₂ emissions and the bio-chemical properties of the soil increased as the aggregate
311 size decreased across the different N additions levels at both forest sites (Figs. 1 and 2, Tables
312 and 4). These results were in agreement with [Kimura et al. \(2012\)](#), who suggested that
313 smaller micro-aggregates contained more easily decomposed organic matter with improved
314 availability for microorganisms. However, macro-aggregates were known to contain more
315 labile organic matter as bonding agents between the micro-aggregates , while C enclosed in
316 micro-aggregates was more stable due to a strong combination with soil particles . These
317 differences in the bio-chemical properties of aggregates were supported by the results from
318 [Elliott et al. \(1996\)](#), who found higher CO₂ emissions from macro-aggregates than micro-
319 aggregates. [Mangalassery et al. \(2013\)](#) also found similar trends in sandy loam texture soil,
320 but the opposite effects in clay loam soil. The above studies suggested that the difference in
321 CO₂ emissions of the different aggregate sizes was attributed to the various nutrient contents
322 in aggregates regardless of soil texture. Therefore, our results found higher CO₂ emissions in
323 smaller aggregates because of higher nutrient contents than larger aggregates (Table 2) and
324 suggesting that the soil properties could be a key factor in influencing C mineralization in
325 forest soils.

326 Nitrogen addition has been reported to increase , decrease and have no effect on CO₂

327emissions from soil due to different control factors (e.g., roots or soil organisms). Examining
328soil from the same area as was used in our study, [Wu et al. \(2015\)](#) also found that both NH_4Cl
329and KNO_3 addition significantly decreased the CO_2 emissions by 9.2-21.6%, and they
330attributed this decrease to decreases in C utilization efficiency by microorganisms in the soil.
331Similarly, [in the present study](#) a decreased trend in CO_2 emissions was observed after N
332addition at both forest sites across all aggregate sizes, but this trend was statistically
333significant only in the PF soil (~30%). [This trend was associated with the decreased of](#)
334[microbial biomass after N addition \(Table 2\). Similarly \[Tao and Song \\(2013\\)\]\(#\) also reported a](#)
335reduction of C mineralization under N addition due to suppressing microbial biomass. These
336results suggest that N addition can influence the sink potential in the mixed Korean pine
337forest.

338

3394.2 CH_4 uptake

340 CH_4 flux from the soil is dependent on the redox conditions, and soils under anaerobic
341conditions promote CH_4 formation; however, under aerobic conditions, CH_4 oxidation is
342favored. In the present study, soil aggregates at both forest sites acted as a sink for CH_4
343during incubation except for the initial days (Figs. 1 and 2). This result [suggested](#) that the
344conditions of all the samples during incubation were favored by aerobic CH_4 oxidation.

345 In present study, the effect of aggregate size on CH_4 emissions differed with forest types.
346In the SF, CH_4 uptake was higher in smaller aggregates, but the pattern in the PF was less
347clear. The higher oxidation of CH_4 in the smaller aggregates may result from aerobic
348conditions and higher resource/nutrient content for microbe metabolism in the SF [with the](#)
349[evidence of the relationship between \$\text{CH}_4\$ oxidation and most of soil properties \(DOC, SOC,](#)
350 [\$\text{NH}_4^+\$ and \$\text{NO}_3^-\$ \). However, less obvious correlation was found between \$\text{CH}_4\$ oxidation and soil](#)
351[properties in the PF \(only MBC was marginally significantly correlated to \$\text{CH}_4\$ oxidation,](#)
352 [\$P=0.077\$ \), indicating that soil properties did not directly influence \$\text{CH}_4\$ oxidation in the PF.](#)
353The small aggregates should confer increased CH_4 emissions because of the reduction in
354ventilation (boosting anaerobic conditions) in repacked small soil aggregates. [Furthermore,](#)

355some studies also suggested that anaerobic conditions can be found in the larger macro-
356aggregates, when fine pore was filled by water after wetting procedure . These characteristic
357of the soil aggregates may be the reasons for the less difference of CH₄ oxidation between
358aggregate sizes in the PF.

359 Evidence suggests that soil N status is one of the major factors that influence CH₄
360oxidation (see reviewed by [Shrestha et al. \(2015\)](#)). In our study, soil N, including NH₄⁺and
361NO₃⁻, was significantly and negatively correlated to the CH₄ uptake rates (Table 5), indicating
362that higher N content limits CH₄ oxidation. Although the results were not significant, the
363inhibitory effect of N addition on CH₄ uptake was observed only in the PF across all
364aggregate sizes (Figs. 1 and 2). However, in the SF, CH₄ uptake rate on a per SOM basis in
365the control plots was similar to the N addition plots, suggesting that higher microorganism
366activity in the broad-leaved forest soil (high content of MBC) suppresses the effect of N
367addition on CH₄ oxidation. These results were in agreement with studies that have shown N
368addition decreased CH₄ oxidation through inhibiting the enzyme activity of CH₄ mono-
369oxygenase . However, some studies have shown positive effects of N addition on soil CH₄
370oxidation , implying that N may be an essential nutrient for methanotrophs to assimilate CH₄
371(Table 5; . At the same site, [Li et al. \(2013\)](#) also reported a slight inhibitory effect of N
372addition on the CH₄ oxidation of bulk soil. Furthermore, a similar incubation study was
373conducted by [Plaza-Bonilla et al. \(2014\)](#), who also found a slight decrease in CH₄ oxidation in
374intact macro-aggregates under fertilized treatment compared to control. These results suggest
375that the addition of N may have negative effect on CH₄ oxidation under mixed Korean pine
376forest rather than broad-leaved forest.

377

3784.3 N₂O emission

379 N₂O emissions have been reported to significantly correlate to soil moisture status and
380quickly reach the maximum emission rate after soil moistening . This initial flush is thought
381to be a result of a rapid change from aerobic to anaerobic conditions and the concomitant
382rapid change in microbial activity after soil moistening. In the present study, increasing N₂O

383emissions were observed with decreasing aggregate size across the two forest sites, with the
384exception of the largest aggregates where cumulative N₂O emissions were higher than in other
385macro-aggregates (Figs. 1 and 2). This agrees with the findings by [Bandyopadhyay and Lal](#)
386(2014) and [Plaza-Bonilla et al. \(2014\)](#), who attributed the higher emissions of N₂O in larger
387macro-aggregates to the presence of anaerobic microsites within the macro-aggregates, which
388also exist in the micro-aggregates. The highest cumulative N₂O emissions in this study were
389recorded in the micro-aggregates with the highest nutrient content (e.g. NH₄⁺, NO₃⁻) at both
390forest sites (Table 2).

391 The effects of N addition on soil N₂O emissions were mostly positive for the field
392observations. Additionally, similar results were obtained from the same sites used in this
393study by [Bai et al. \(2014\)](#), who reported a positive effect of laboratory N addition on N₂O
394emission. Few studies have failed to observe a significant increase in N₂O emissions after N
395addition, which was attributed to competition for N by trees. Furthermore, there has been
396limited investigation in the effects of long-term N addition on N₂O emission in aggregates. In
397our experiment, no difference in N₂O emissions was recorded between N addition treatments
398in the SF, while a negative effect was found in the PF, especially scaling down to per SOM
399basis ($P=0.024$). The N₂O emissions in the SF was correlated with most of soil properties
400determined in this study, while they only significant correlated with DOC and NO₃⁻ in the PF
401(Table 5). These results indicated that soil properties in the SF was easier for microbial
402utilization than in the PF, and therefore, resulting in different responses of N₂O emissions to N
403addition.

404

4054.4 GWP

406To compare the total effect of N addition on GHG emissions of the two forest sites, the
407radiative force from the net GHG emissions was calculated using the GWP (Fig. 3). The CO₂
408emissions were the greatest contributor to the GWP (> 90%), while CH₄ negatively
409contributed to the GWP. This finding was in agreement with [Bandyopadhyay and Lal \(2014\)](#)
410and [Shrestha et al. \(2014\)](#) who also found that CO₂ was the largest contributor to the GWP

411(98.9% in forest soil). However, [Mosier et al. \(2005\)](#) found that N₂O emissions accounted for
41240-44% of GWP in an agricultural soil, whereas it only accounted for 1% in present study.
413The different patterns of GWP between agricultural soil and forest soil can be attributed to
414agricultural production practices (e.g. tillage, irrigation or fertilization etc.) . For N addition
415treatments, the decreased trend of GWP after N addition mainly resulted from the reduction of
416CO₂ emissions after N addition due to the highest contributions of CO₂ to GWP (Fig. 3). But
417several studies including agricultural soil and forest soil reported a positive effect of N
418addition on GWP in a field investigations. Whereas, no effects of N addition on GWP were
419also reported [Jiang et al. \(2010\)](#). These different effects of N addition on GWP can be
420attributed to the differences in soils (agricultural soils or forest soils), N addition treatment
421durations (short-term or long-term), and fertilizer types (inorganic N or organic N). Therefore,
422the result in our study suggested that N addition may decrease the GHG emissions and
423subsequently increase C sequestration in the soil, particularly in mixed Korean pine forests.

424

4255 Conclusions

426The GHG emissions from the temperate forest soil were influenced by several factors.
427Aggregate size and N addition had a significant influence on releases of GHGs from soils.
428The emission/uptake rates of GHGs in the micro-aggregates were higher than that in the
429macro-aggregates at both forest sites due to contained more resources in micro-aggregates
430than macro-aggregates. The addition of N to the soil significantly inhibited the GHG
431emission/uptake rates in the mixed Korean pine forest soil (PF), while the effect was minimal
432in the broad-leaved forest (SF). These results indicated that aggregate size and N addition
433have profound implications on GHG emissions but differed in forest types. The GWP was
434increased with aggregate size decreased at both forest sites. The effect of N addition on GWP
435was negative in the mixed Korean pine forest soil, but less clear in the broad-leaved forest
436soil. These results suggested that N addition decreased the emissions of GHGs depending on
437the forest type and the various aggregates, and therefore, may increase C sequestration in the
438mixed Korean pine forest.

439

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443

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598

599

600**Tables**

601

602**Table 1** Soil characteristics at the two forest sites (SF = secondary forest; PF = primary forest)

	pH	SOC (%)	TN (%)	C/N	NH ₄ ⁺ (mg kg ⁻¹)	NO ₃ ⁻ (mg kg ⁻¹)	soil texture (%)		
							sand	silt	clay
SF	5.20	22.54	1.71	13.10	21.37	47.10	26	40	34
PF	5.16	15.60	1.03	15.01	31.39	27.18	10	51	38

603Data (Mean) were determined from the bulk soil derived from Lou (unpublished data, 2013).

604 **Table 2** Soil bio-chemical properties under different N addition treatments (N0 vs. N50) and
605 aggregate size classes (mm) at the two forest sites (SF = secondary forest; PF = primary forest)

treatment	NH ₄ ⁺ (mg kg ⁻¹)		NO ₃ ⁻ (mg kg ⁻¹)		DOC (mg kg ⁻¹)		MBC (mg kg ⁻¹)		SOC (g kg ⁻¹)	TN (g kg ⁻¹)
	before	after	before	after	before	after	before	after	before	before
SF-N0										
4-8 mm	112.9 0	339.1 2	6.19	25.10	827.28	1516.1 1	2256.7 4	1598.7 4	124.55	11.00
2-4 mm	161.7 5	329.7 3	9.23	17.10	814.11	1643.9 0	2075.5 5	1273.1 3	124.78	10.56
1-2 mm	161.3 1	328.7 3	12.96	17.64	951.96	1966.0 8	2321.5 1	1660.4 6	116.50	10.02
0.25-1 mm	142.4 0	342.8 1	12.63	19.84	1059.5 3	2036.5 2	2176.9 5	1526.5 8	128.18	10.92
free-m	196.1 5	405.8 8	25.10	34.38	1253.4 9	2686.0 2	2421.6 3	2069.6 5	130.96	11.72
SF-N50										
4-8 mm	94.00	322.1 0	13.37	17.93	756.87	1633.2 3	2241.2 8	1152.0 3	126.02	10.86
2-4 mm	109.9 0	332.9 1	11.74	18.54	655.02	1369.5 6	1988.1 8	1355.5 4	127.77	10.80
1-2 mm	131.7 0	360.4 6	12.25	16.06	666.09	1463.3 8	2250.7 0	1541.3 6	128.87	10.62
0.25-1 mm	144.9 3	373.6 7	14.27	16.65	793.44	1624.0 3	2446.2 0	1472.5 1	137.80	11.34
free-m	179.4 2	385.0 5	27.97	50.84	1129.1 2	2673.1 7	3765.6 1	1951.8 6	139.89	10.86
<i>P</i> -value of ANOVA of each soil properties in SF										
S	0.240	0.009	0.000	0.002	0.037	0.000	0.111	0.523	0.354	0.666
N	0.310	0.626	0.101	0.762	0.067	0.131	0.230	0.616	0.621	0.915
S × N	0.954	0.432	0.622	0.386	0.856	0.584	0.338	0.974	0.940	0.902
PF-N0										
4-8 mm	305.7 4	280.5 2	106.9 0	127.9 4	739.25	1633.2 8	1618.7 0	1633.2 8	104.67	7.70
2-4 mm	377.3 4	281.9 2	117.2 8	140.1 1	709.47	1575.0 5	1860.4 5	1575.0 5	135.37	9.30
1-2 mm	379.7 1	299.2 7	130.9 2	137.3 3	859.70	1462.7 4	3028.7 5	1462.7 4	150.50	10.07
0.25-1 mm	389.0 9	309.8 1	131.7 4	131.5 7	933.50	1770.5 6	3301.6 7	1770.5 6	154.43	10.47
free-m	439.0 5	392.2 9	219.9 7	173.0 4	1342.7 9	2731.1 3	3755.8 6	2731.1 3	176.13	11.70
PF-N50										
4-8 mm	226.1 9	264.1 6	164.9 6	98.29	811.95	1415.2 0	2158.2 7	1415.2 0	105.77	7.57
2-4 mm	326.3 2	278.4 3	133.4 5	88.52	858.36	1286.6 0	2210.3 8	1286.6 0	131.50	8.73
1-2 mm	369.2 4	285.8 7	155.4 8	108.3 1	922.91	1378.4 4	2070.7 0	1378.4 4	134.67	8.63
0.25-1 mm	410.0 7	278.2 3	154.2 6	104.0 5	1096.9 8	1629.2 4	2417.6 1	1629.2 4	149.30	9.27
free-m	408.4 8	367.9 2	267.2 4	107.8 5	1425.5 1	2477.7 7	3278.0 4	2477.7 7	158.47	10.30
<i>P</i> -value of ANOVA of soil properties in PF										
S	0.430	0.009	0.168	0.918	0.000	0.000	0.181	0.000	0.190	0.183
N	0.585	0.338	0.307	0.386	0.021	0.201	0.529	0.201	0.421	0.405

S × N		0.981	0.991	0.992	0.812	0.912	0.992	0.747	0.992	0.989	0.992
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The difference in each soil properties before and after incubation (paired t-test)

<i>P</i> -value	in	<0.001	<0.001	<0.001	<0.001
SF					
<i>P</i> -value	in	0.036	0.028	<0.001	<0.001
PF					

606

607 **Table 3** ANOVA results for the effect of aggregate size and N addition on 7-days cumulative GHG
 608 emissions expressed by per aggregate mass at the two forest sites (SF = secondary forest; PF =
 609 primary forest)

Source of Variations	CO ₂			N ₂ O			CH ₄		
	DF	F	<i>P</i>	DF	F	<i>P</i>	DF	F	<i>P</i>
SF									
S	4	4.645	0.002	4	3.709	0.008	4	96.217	0.000
N	1	0.256	0.614	1	0.121	0.729	1	7.466	0.008
S × N	4	0.128	0.972	4	1.145	0.341	4	0.304	0.874
PF									
S	4	5.099	0.002	4	4.000	0.007	4	1.795	0.145
N	1	24.574	0.000	1	1.835	0.182	1	1.579	0.215
S × N	4	0.144	0.965	4	1.298	0.284	4	0.271	0.895

610 S: aggregate size, N: N addition, ×: interaction effect.

611 **Table 4** GHG emission rates (calculated by per SOM) under different N additions (N0 vs. N50)

612 and aggregate size classes (mm) at the two forest sites (SF and PF)

	SF-N0		SF-N50		PF-N0		PF-N50	
	mean	SE	mean	SE	mean	SE	mean	SE
$\text{CO}_2/\text{mg kg}^{-1} \text{ h}^{-1}$								
4-8 mm	377.56 aA	28.63	368.41 aAB	8.58	166.51 aAB	24.41	100.53 bA	10.64
2-4 mm	349.20 aA	16.65	352.31 aAB	14.71	164.03 aAB	17.53	83.72 bA	3.06
1-2 mm	428.90 aB	13.89	383.94 aAB	11.10	154.85 aAB	4.10	93.32 bA	8.03
0.25-1 mm	433.46 aB	17.94	395.65 bA	14.39	139.84 aA	3.43	85.03 bA	9.55
Free-m	359.32 aA	14.73	350.44 aB	9.95	195.37 aB	39.26	105.48 bA	13.97
$\text{N}_2\text{O}/\mu\text{g kg}^{-1} \text{ h}^{-1}$								
4-8 mm	8.96 aB	1.80	10.59 aA	2.52	7.28 aA	1.39	8.18 aA	1.55
2-4 mm	6.97 aB	0.69	7.80 aA	0.94	7.45 aA	2.08	4.19 aB	0.69
1-2 mm	8.82 aB	0.58	8.12 aA	0.54	6.76 aA	1.14	4.48	0.45
0.25-1 mm	8.64 aB	0.53	8.37 aA	0.99	5.59 aA	1.17	aAB 4.45	0.32
Free-m	13.40 aA	2.45	9.96 aA	0.64	9.24 aA	2.30	aAB 5.26 bAB	0.40
$\text{CH}_4/\mu\text{g kg}^{-1} \text{ h}^{-1}$								
4-8 mm	-11.58 aC	0.93	-12.88 aC	0.67	-7.82 aA	1.62	-5.67	1.14
2-4 mm	-13.41 aC	1.07	-17.38 aBC	0.99	-7.33 aA	1.58	aA -4.18	1.64
1-2 mm	-19.69 aB	0.75	-20.26 aB	0.79	-3.75 aA	0.58	aA -3.99	0.49
0.25-1 mm	-18.45 aB	1.14	-18.76 aB	1.46	-3.61 aA	0.88	aA -2.42	0.81
free-m	-33.16 aA	2.40	-39.24 bA	3.48	-7.18 aA	2.63	aA -4.21	0.44

613 Different lowercases indicated difference between N addition treatments in each aggregate size

614 was determined. Different uppercases indicated difference between aggregate sizes in each N

615 addition treatments was determined.

616**Table 5** Relationships (Pearson's correlation coefficient, *r*) between soil initial properties (before
617incubation) and GHG emission rates in each forest site (n=30)

	N ₂ O	CH ₄	MBC	DOC	NH ₄ ⁺	NO ₃ ⁻	SOC	TN
SF								
CO ₂	0.609**	-0.156	0.418*	0.482**	0.360*	0.180	0.604**	0.763**
N ₂ O	1	-0.375*	0.323	0.709**	0.345*	0.472**	0.651**	0.757**
CH ₄		1	-0.204	-0.669**	-0.532**	-0.776**	-0.472**	-0.238
MBC			1	0.585**	0.363*	0.475**	0.182	0.332*
DOC				1	0.576**	0.738**	0.588**	0.570**
NH ₄ ⁺					1	0.579**	0.448**	0.350*
NO ₃ ⁻						1	0.471**	0.324
SOC							1	0.869**
PF								
CO ₂	0.276	-0.447*	0.440*	0.313	0.168	-0.246	0.502**	0.550**
N ₂ O	1	-0.236	0.182	0.445*	-0.158	0.479**	-0.212	-0.126
CH ₄		1	-0.327	-0.210	-0.080	0.066	0.052	-0.117
MBC			1	0.687**	0.241	-0.125	0.583**	0.686**
DOC				1	0.491**	0.302	0.348*	0.363*
NH ₄ ⁺					1	0.300	0.448**	0.426**
NO ₃ ⁻						1	-0.218	-0.220
SOC							1	0.954**

618**P*<0.05; ***P*<0.01

619 Figure Captions

620 **Fig. 1** Cumulative emission of CO₂, N₂O and CH₄ as affected by aggregate sizes (4-8 mm, 2-4
621 mm, 1-2 mm, 0.25-1 mm and free-m) and N addition (N0 = 0 kg N ha⁻¹ yr⁻¹; N50 = 50 kg N ha⁻¹ yr⁻¹)
622) at the secondary forest (SF). Data was presented by mean ± standard error. The dash line
623 presented the zero point.

624

625 **Fig. 2** Cumulative emission of CO₂, N₂O and CH₄ as affected by aggregate sizes (4-8 mm, 2-4
626 mm, 1-2 mm, 0.25-1 mm and free-m) and N addition treatments (N0 = 0 kg N ha⁻¹ yr⁻¹; N50 = 50
627 kg N ha⁻¹ yr⁻¹) at the primary forest (PF). Data was presented by mean ± standard error. The dash
628 line presented the zero point.

629

630 **Fig. 3** Global warming potential (GWP) as affected by aggregate sizes (4-8 mm, 2-4 mm, 1-2 mm,
631 0.25-1 mm and free-m), N addition treatments (N0 = 0 kg N ha⁻¹ yr⁻¹ and N50 = 50 kg N ha⁻¹ yr⁻¹)
632 and forest types (secondary forest, SF and primary forest, PF). Data was presented by mean ±
633 standard error. Different uppercases mean the significant difference between aggregate sizes was
634 determined across N addition treatments. Different lowercases mean the significant difference
635 between N addition treatments was determined.

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