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Effects of nitrification inhibitors on gross N nitrification rate, ammonia oxidizers, and N₂O production under different temperatures in two pasture soils

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4 **Title:** Effects of nitrification inhibitors on gross N nitrification rate, ammonia
5 oxidizers, and N₂O production under different temperatures in two pasture soils

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22 Abstract

23 Australian pasture soil for cattle and sheep industries constitute the principal land use with
24 considerable N fertilizer consumption, which is one of the causes of local environmental
25 problems. Nitrification plays a key role in regulating soil inorganic N concentration and its
26 environmental diffusion. The effects of different nitrification inhibitors (NIs) on gross N
27 nitrification (n_{gross}) rate and N₂O production under different temperatures in pasture soils
28 remain unclear. A laboratory incubation experiment was conducted to determine the effect of
29 NIs (dicyandiamide [DCD], 3,4-dimethylpyrazole phosphate [DMPP], and 3-methylpyrazol
30 and 1H-1,2,4-triazol [3MP+TZ]) on N₂O emissions, n_{gross} and net N nitrification (n_{net}) rates,
31 and the abundance of ammonia oxidizers, namely, ammonia-oxidizing archaea (AOA) and
32 ammonia-oxidizing bacteria (AOB), in two Australian pasture soils incubated at temperatures
33 of 15 °C, 25 °C and 35 °C. All NIs reduced both n_{gross} and n_{net} rates and N₂O production rate
34 from the two pasture soils but to different extents. The inhibitory rates of NIs on n_{gross} and n_{net}
35 reached 6.80%–63.8% and 5.91%–62.3%, respectively, whereas that on N₂O production rate
36 totaled 4.5%–41.4% in the tested soils. NIs reduced nitrification and N₂O production by
37 inhibiting the growth of AOB rather than AOA. The inhibitory effects of NIs were
38 temperature-dependent, that is, decreasing with increasing temperature from 15 °C to 35 °C.
39 In general, DMPP performed better than DCD and 3MP+TZ at 15 °C and 35 °C, whereas
40 DCD performed more effectively than the other two NIs at 25 °C. Our results suggest that the
41 utilization of NIs will depend on the conditions present, especially soil temperature.
42 Additionally, AOB is the target of inhibition when mitigating nitrification and N₂O emission
43 by applying NIs in pasture soils.

44 **Keywords:** Acid soils · AOA · AOB · Gross N nitrification rate · ¹⁵N dilution

45 **Introduction**

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3 46 Nitrification is a microbial process that plays important roles in regulating soil
4
5 47 inorganic N concentration, NO_3^- leaching, and N_2O production (Bremner 1997). N_2O
6
7 48 is a potent greenhouse gas that also contributes to stratospheric ozone destruction
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10 49 (IPCC 2007). Nitrification can be either autotrophic or heterotrophic. Ammonia-
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12 50 oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) are involved in the
13
14 51 first step of autotrophic nitrification, that is, the oxidation of NH_3 to NO_2^- in soil
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16 52 (Francis et al. 2005). AOA and AOB can be identified by the *amoA* gene, which is a
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18 53 functional marker encoding the ammonia mono-oxygenase (AMO) enzyme (McCarty
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20 54 1999). Conversely, few heterotrophs carry out heterotrophic nitrification (oxidize
21
22 55 either NH_3 or organic N to form NO_3^-) in soil, usually at much lower rates than those
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24 56 carried out by autotrophs (Sahrawat et al. 2008). However, complete ammonia
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26 57 oxidizers within the *Nitrospira* genus, which can convert ammonia to nitrate in a
27
28 58 single organism (comammox), were discovered (Hu and He 2017).

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34 59 Nitrification inhibitors (NIs) can decelerate the rate of soil nitrification by
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36 60 deactivating AMO and blocking the first and rate-limiting steps of autotrophic
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38 61 nitrification, specifically, NH_3 oxidization (Di et al. 2009; Di and Cameron, 2012). By
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40 62 suppressing autotrophic nitrification, NIs potentially reduce subsequent denitrification
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42 63 and N leaching. As a result, reduction in the N_2O produced through this pathway is
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44 64 expected to occur (Bauhus et al. 1996; Lan et al. 2013; McTaggart et al. 1997). A
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46 65 meta-analysis by Akiyama et al. (2010) showed that compared with conventional
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48 66 fertilizers application of NIs reduced N_2O emissions by 44% to 31%, with an average
49
50 67 of 38%. Dicyandiamide (DCD, $\text{C}_2\text{H}_4\text{N}_4$) and 3,4-dimethylpyrazole phosphate (DMPP)
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52 68 are the two most commonly used NIs. DCD has been studied for over 80 years, and its
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54 69 effect in reducing both NO_3^- leaching and N_2O emission has been reported for

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70 different agroecosystems, such as maize–wheat crop systems (Sharma and Prasad
71 1996), grazed pastures (Di and Cameron 2004; 2012), and paddy soils (Kumar et al.
72 2000; Lan et al. 2013). DMPP was demonstrated as one of the most efficient NIs to
73 date and has undergone ecotoxicological and standard toxicological tests (Menendez
74 et al. 2012; Benckiser et al. 2013). Zerulla et al. (2001) specified several
75 advantageous properties of DMPP compared with DCD. For example, DMPP can be
76 used with solid fertilizer granules at a lower rate than that of DCD. 3-Methylpyrazol +
77 1H-1,2,4-triazol (3MP+TZ) is a synthetic compound proposed as a NI (Hu et al.
78 2013). However, this compound is less commercially used than DCD and DMPP, and
79 its inhibitory effects on nitrification or N₂O emission are unclear.

80 Evidence showed that the effect of NIs in reducing nitrification rate produce
81 inconsistent results and is affected by environmental factors, such as soil temperature.
82 For example, Merino et al. (2005) observed that inhibition by DMPP can last longer
83 in autumn than in spring. Menéndez et al. (2012) discovered a declining inhibitory
84 efficacy of DMPP with increasing soil temperature from 10 °C to 20 °C. According to
85 Di and Cameron (2014), the half-life of DCD increased from 111 days to 116 days
86 under a soil temperature of 8 °C but shortened to 18–25 days when the soil
87 temperature reached 20 °C. These observations suggest that high soil temperatures
88 can lower the NIs efficiency owing to the faster molecular degradation under these
89 conditions. Therefore, soil temperature is an important factor affecting NI efficacy in
90 systems with repeated NI applications.

91 The agricultural sector of Australia contributes 16% of the total greenhouse gas
92 emissions, contributing approximately 85% of all N₂O emissions (20.7 Mt CO₂-eq.)
93 (AGO 2010). Pasture soils for sheep and cattle industries constitute the principal land
94 use and covers approximately 450 million ha of the lands in Australia (AGO 2010);

1 95 the industry is considered the largest N fertilizer user in the country. Previous studies
2
3 96 have demonstrated that applying NIs can effectively reduce autotrophic nitrification
4
5 97 and N₂O emissions from pasture soils (Friedl et al. 2017). For example, Di et al.
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7 98 (2016) reviewed the effects of DCD and DMPP in inhibiting NO₃⁻ leaching and N₂O
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9 99 emission in pasture soils, and the results showed that N₂O emission from animal
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12 100 urine-N can be reduced by 57%. Evidence showed that N₂O emission is significantly
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14 101 related to the amount of NO₃⁻-N in pasture soils and to *amoA* gene copy numbers of
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16 102 AOB but not related to the population of AOA (Di et al. 2009). In addition, both AOB
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18 103 population growth and the amounts of soil NO₃⁻-N concentration were significantly
19
20 104 related to the amount of DCD application. However, AOA population abundance
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22 105 showed no relationship to the application rate of DCD (Guo et al. 2014). Although
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24 106 various studies have investigated the effect of different NIs on nitrification rate, N₂O
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26 107 emission, and ammonia oxidizers in pasture soils, the influence of temperature on the
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28 108 inhibitory efficacy of NIs in pasture soils with low pH and high organic C content
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30 109 remains unclear. Evidence showed that heterotrophic nitrification, a process that
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32 110 cannot be inhibited by NIs, may act as the predominant pathway in producing NO₃⁻ in
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34 111 soils at low pH and high recalcitrant organic C primarily in forest ecosystems and
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36 112 grassland (Müller et al. 2004; Zhang et al. 2013). In addition, most previous studies
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38 113 have been carried out by measuring the net changes in NH₄⁺-N and NO₃⁻-N
39
40 114 concentrations in pasture soils. Net rate results from several opposing soil processes.
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42 115 We possess very limited knowledge about the effectiveness of different NIs in *n*_{gross}
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44 116 rate of pasture soils. The gross rates of NH₄⁺ and NO₃⁻ production measured using the
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46 117 ¹⁵N isotopic pool dilution technique provide considerable information about N
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48 118 transformation processes than net rates (Müller et al. 2004). However, the effects of
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50 119 temperature on the efficacy of NIs in inhibiting ammonia oxidizers (AOA and AOB)

120 in pasture soils remain unclear.

121 This study was designed to verify the following points: (i) the effects of NIs
122 (DCD, DMPP, and 3MP+TZ) on n_{net} and n_{gross} rates, ammonia oxidizers (AOA and
123 AOB), and N_2O production from pasture soils with low pH and high organic C
124 content; (ii) the effects of temperature on the efficacy of NIs. We hypothesized that (i)
125 NIs can only partly inhibit nitrification and N_2O production in pasture soils with low
126 pH and high organic C content; (ii) the efficacy of NIs on n_{gross} rate in pasture may
127 vary under different temperatures. The findings from the study can improve our
128 understanding of the interactions of NIs with nitrification rate, N_2O production, and
129 soil microbial communities in pasture soils under a range of temperatures.

131 **Materials and methods**

132 **Soil collection**

133 Two pasture soils were used in the incubation experiments. Soils were collected from
134 two typical pasture lands, namely, Glenormiston (GN; 38.18° S, 142.97° E) and
135 Terang in Victoria (TR; 33.73° S, 84.43° E), Australia. Soil samples were randomly
136 collected from 10 locations to a depth of 15 cm. The collected samples were mixed
137 into one composite sample. Soil samples were thoroughly homogenized, stones and
138 plant roots were removed by hand, and the soil was sieved at <2 mm mesh before
139 being air dried. Table 1 shows the physical and chemical properties of the two tested
140 pasture soils.

142 **Soil incubation experiment**

143 A total of 60 g (dry weight) soil samples were introduced into a 500 ml plastic vial.
144 To reactivate soil microorganisms, we rehydrated the soil and preincubated it at 15 °C,

145 25 °C, or 35 °C for 21 days. The following five treatments (each with four replicates)

146 were included in this study:

147 1) Control (equivalent water, no fertilizer) (abbr.: control)

148 2) $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3$ (containing 26% N) (abbr.: ACl)

149 3) $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3+\text{DMPP}$ (DMPP at 0.7% of applied NH_4^+-N) (abbr.: DMPP)

150 4) $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3+3\text{MP}+\text{TZ}$ (3MP [at 0.33%]+TZ [at 0.67%] of applied NH_4^+-

151 N), (abbr.: 3MP+TZ)

152 5) $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3+\text{DCD}$ (DCD at 4.5% of applied NH_4^+-N) (abbr.: DCD)

153 A total of 600 vials (5 treatments \times 4 replicates \times 3 temperature \times 5 destructively

154 sampling time \times 2 soils = 600 vials) were observed. The application rates of NIs were

155 typical of the amount applied to pasture soils (Di et al. 2004; Di and Cameron 2012;

156 Friedl et al. 2017). Fertilizer treatments were surface-applied to the soil on 0-day. The

157 application rate was $100 \text{ mg kg}^{-1} \text{ NH}_4^+-\text{N}$ plus $50 \text{ mg NO}_3^--\text{N kg}^{-1} \text{ soil}$, and the ^{15}N

158 atom% of NO_3^- was 10%. An equivalent volume of deionized water (instead of NIs

159 and fertilizer) was applied on the control treatment, and soil water content of all

160 treatments was adjusted to 60% water-filled pore space. Immediately after application,

161 all vials were covered with parafilm, and four pin holes were created to facilitate air

162 exchange. Vials were incubated at 15 °C, 25 °C and 35 °C for 28-day. Vials were

163 weighed every two days to calculate water loss, and deionized water was replenished

164 as needed.

165

166 **Gas collection and analysis**

167 Headspace gas was obtained from the 500 ml vials using gas-tight syringes 0, 1, 2, 3,

168 4, 7, 10, 14, 21, and 28 days after applying N fertilizer and NIs. Prior to each gas

169 collection, the air in vials was replaced with zero air (pure N₂) to balance the air
170 pressure when performing gas sampling. On each sampling day, 20 ml gas samples
171 were collected 0 and 12 h after closing the vials with a plastic syringe and storing
172 them into a pre-evacuated exetainer (Exetainer[®], Labco Ltd., Lampeter, Ceredigion,
173 UK). Samples were analyzed for N₂O concentration by Agilent 7890 Gas
174 Chromatograph equipped with ECD detector.

175

176 **Soil collection and analysis**

177 Soils were destructively sampled for the analysis of mineral N (NH₄⁺ and NO₃⁻)
178 concentration and their ¹⁵N isotopic abundance immediately after gas sampling on
179 days 0, 7, 14, 21, and 28. A total of 2 g soil subsamples were obtained on days 0, 14,
180 and 28 from each vial for molecular analysis and stored at -80 °C prior to DNA
181 extraction. The remaining soil was extracted with 2 M KCl (soil:solution=1:5) by
182 shaking for 1 h, and KCl extracts were filtered through a quantitative filter paper.
183 Extract samples were maintained at -20 °C until analysis for mineral N with a
184 continuous-flow autoanalyzer (Skalar, SAN++) and ¹⁵N abundance in mineral N (100
185 ml) after microdiffusion. The detailed procedure of the microdiffusion methods has
186 been reported by Saghir et al. (1993) and Liu et al (2015a, b). The isotope
187 composition of mineral N was performed by isotope-ratio mass spectrometry (Crewe,
188 UK). The abundances of AOA and AOB *amoA* genes were quantified by real-time
189 quantitative polymerase chain reaction (qPCR) with primers targeting the archaeal
190 and bacterial *amoA* genes (Francis et al. 2005). Details of the methods of soil DNA
191 extraction and qPCR analysis have been reported (Shi et al. 2016).

192 **Calculations**

193 The n_{net} rates during the 28-day incubation were calculated from the changes in NO_3^-
194 concentrations by using the following equation:

$$195 \quad n_{\text{net}} = ([\text{NO}_3^- - \text{N}]_{t_1} - [\text{NO}_3^- - \text{N}]_{t_0}) / (t_1 - t_0)$$

196 where t represents the incubation time (day).

197 According to the principle of ^{15}N dilution, n_{gross} rates were calculated using the
198 data obtained in 0–7 day of incubation. This process was performed as soil N
199 transformation will complicate if the incubation time exceeds 7-day, processes such as
200 remineralization will occur, thereby leading to inaccuracy in n_{gross} rates. The classical
201 ^{15}N isotopic dilution equation was used to calculate the n_{gross} rates (Kirkham and
202 Bartholomew 1954).

$$203 \quad n = \frac{[(\text{NT}_1 - \text{NT}_2) / \Delta t] \log(\text{NL}_1 \text{NT}_2 / \text{NL}_2 \text{NT}_1)}{\log(\text{NT}_1 / \text{NT}_2)}$$

204 where NL represents labeled NO_3^- ; NT denotes the total NO_3^- (labeled+unlabeled);
205 Δt is the time interval. Subscripts 1 and 2 denote the 0- and 7-day values,
206 respectively.

207 N_2O fluxes were calculated according to the following equation:

$$208 \quad F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + T}$$

209 where F is the gas flux in $\text{ng N}_2\text{O-N m}^2 \text{ h}^{-1}$, ρ stands for the density of N_2O in the
210 standard state (g ml^{-1}), V species the volume of the head space (ml), A is the area of
211 the vial (cm^2), $\Delta c / \Delta t$ indicates the change in N_2O concentration per unit of time
212 (ppm d^{-1}), and T is the air temperature within the vials ($^{\circ}\text{C}$).

213 **Statistical analyses**

214 Statistical analyses were performed using SPSS 18.0 software. One-way ANOVA was
215 used to compare differences in the average rates of N_2O flux, n_{net} and n_{gross} among the

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216 different treatments ($P<0.05$). The relationships of n_{net} rate and NO_3^- -N concentration
217 to N_2O production rate were investigated by redundancy and correlation analyses.

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219 **Results**

220 **Mineral N**

221 In the control treatments, the exchangeable NH_4^+ -N concentrations increased with the
222 increase in incubation time for both soils at three temperatures (Figs. 1 and 2).
223 However, in ACI treatments, the concentration of exchangeable NH_4^+ decreased with
224 prolonged incubation, showing a decline toward the control levels within four weeks
225 in both soils (Figs. 1 and 2). The amendment of NIs retarded the decrease in NH_4^+
226 concentrations, and such effects were more evident at 15 °C than at 25 °C and 35 °C.
227 Compared with the control treatment, adding NH_4Cl significantly increased the
228 NO_3^- -N concentrations over time ($P<0.05$), indicating nitrification occurrence. NO_3^-
229 concentration increased more slowly at low temperature (Figs. 1 and 2), that is, 15 °C,
230 than at high temperature, that is, 35 °C (Figs. 1 and 2). The amendment of NIs
231 significantly retarded the accumulation of NO_3^- -N in both soils, and such effects
232 were more significant in DMPP treatment than in DCD and 3MP+TZ, regardless of
233 the temperature treatment and soil used (Figs. 1 and 2).

234

235 **Nitrification rate**

236 Fig. 3 shows the n_{gross} rates at days 0–7 and the time-weighted averages of n_{net} rates
237 during the 28-day incubation. ACI treatments yielded the highest n_{net} values in both
238 soils (2.94–5.95 and 1.31–2.32 $\text{mg kg}^{-1} \text{day}^{-1}$ in GN and TR soil, respectively). All
239 NIs also significantly inhibited the n_{net} rates, with inhibition rates varying from 5.91%
240 to 62.3% (Fig. 3). In general, the inhibitory effect of NIs on n_{net} rates decreased with

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241 increasing soil temperature regardless of the NI type. In GN soil, DMPP performed
242 better than DCD and 3MP+TZ under the three temperatures (Fig. 3). However, no
243 statistical difference in n_{net} was detected between DMPP, DCD, and 3MP+TZ at the
244 three temperatures in TR soil ($P>0.05$, Fig. 3).

245 The n_{gross} rates of ACI treatments (7.12–15.2 and 9.82–12.4 mg kg⁻¹ d⁻¹ in GN
246 and TR soil, respectively) significantly increased when the temperature was increased
247 from 15 °C to 25 °C in both GN and TR soils ($P<0.05$, Fig. 3). However, no
248 difference in n_{gross} rate was observed between 25 °C and 35 °C ($P>0.05$, Fig. 3). The
249 n_{gross} rates were in agreement with the rapid consumption of NH₄⁺ in soil at 25 °C and
250 35 °C, and they were generally higher in GN soil than in TR soil during incubation at
251 the same temperature (Fig. 3). The n_{gross} rates were inhibited by NIs from 6.80% to
252 63.8% (Fig. 3), although the inhibitory effects of DMPP, DCD, and 3MP+TZ on n_{gross}
253 rate declined with increasing soil temperature from 15 °C to 35 °C. DMPP worked
254 better than DCD and 3MP+TZ in both GN and TR soils at 15 °C and 35 °C, but DCD
255 presented the best performance among the studied NIs at 25 °C (Fig. 3).

256 257 **N₂O production rate**

258 Soil temperature and NI application significantly affected the average daily N₂O
259 production rates in both GN and TR soils (Fig. 4). The N₂O production rates over the
260 28-day incubation time increased with increasing soil temperature, and the highest
261 N₂O production rates were observed in ACI treatment regardless of the temperature
262 and soil sample used (Fig. 4). N₂O production was reduced by 23.9%–42.6%, 16.3%–
263 41.4%, and 13%–27.8% in GN soil and by 4.5%–11.6%, 20.3%–25.3%, and 20.7%–
264 25.8% in TR soil by applying DCD, DMPP, and 3MP+TZ, respectively. However, the

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265 inhibitory effects of NIs were affected by incubation temperature. The highest
266 inhibition effect by DCD was achieved at 25 °C and 35 °C in GN and TR soil,
267 respectively. On the other hand, the highest inhibition effects of DMPP and 3MP+TZ
268 were achieved at 35 °C and 25 °C for both soils, respectively.

269

270 **AOA and AOB populations**

271 In GN soil, both AOA and AOB *amoA* gene copy numbers increased with incubation
272 time but decreased as incubation temperature increased from 15 °C to 35 °C (Fig. 5).
273 The application of NIs affected AOB abundance with respect to the ACI treatment
274 (10.1%–44.8% inhibition rate on 14-day; 10.9%–39.4% inhibition rate on 28-day) but
275 not AOA abundance. Throughout the entire incubation period, the largest average
276 reduction effect of NIs on bacterial *amoA* gene abundance was observed in DMPP
277 treatment at 15 °C (29.1%), followed by DCD and DMPP treatments at 25 °C (25.8%)
278 and 35 °C (11.9%), respectively.

279 In TR soil, both AOA and AOB *amoA* gene copy numbers under ACI treatment
280 increased from 2 h on 14-day and then decreased for the rest of the incubation time.
281 This finding was true except for AOA at 25 °C and AOB at 15 °C, which both
282 decreased from 0-day to 14-day and increased from 14-day to 28-day (Fig. 6). The
283 variation ranges of AOA and AOB *amoA* gene copy numbers during three sampling
284 dates were significantly larger at the incubation temperature of 15 °C than at 25 °C
285 and 35 °C (Fig. 6). Similar to that in GN soil, adding NIs caused no effect on AOA
286 growth in TR soil. This observation was obtained except for DCD and 3MP+TZ
287 treatment at 15 °C on 14-day (Fig. 6). By contrast, applying the three NIs to TR soil
288 significantly inhibited AOB population under 25 °C and 35 °C on 14-day and under
289 15 °C, 25 °C, and 35 °C on 28-day. The highest inhibition effect on AOB population

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290 was achieved at 15 °C in DMPP treatment (42.8%), followed by DCD treatment at
291 25 °C (24.7%) and DMPP treatment at 35 °C (20.2%).

292

293 **Discussion**

294 **Effects of temperature on NIs efficacy**

295 DCD, DMPP, and 3MP+TZ can inhibit the n_{net} and n_{gross} in the two studied pasture
296 soils (Fig. 3). However, soil temperature significantly affected their efficacy. The
297 increased incubation temperature lowered the efficacy of these NIs (Fig. 3). This
298 phenomenon may be due to at least three reasons. The first reason is the positive
299 effect of temperature on soil nitrification activity. Sahrawat (2008) reported that
300 nitrification generally follows a bell-shaped temperature response curve with an
301 optimum temperature ranging from 25 °C to 35 °C. According to Avrahami and
302 Bohannan (2007), nitrification activity intensifies with rising soil temperature, and
303 evidence revealed that temperature negatively affects the abundance of ammonia
304 oxidizers but positively affects their activity (Gubry-Rangin et al. 2017). The second
305 reason is the rapid decomposition of NIs by soil microorganisms when the
306 temperature was increased. Previous studies reported the hastened NI degradation in
307 soil at increased temperatures (Di and Cameron 2014; Menéndez et al. 2012; Puttanna
308 et al. 1999) and accelerated microbiological degradation of NIs with increasing
309 temperature, with the maximum values reached between 25 °C and 33 °C.
310 Additionally, half-life dissipation decrease was higher under laboratory incubation
311 conditions than under field practice conditions because of the presence of other
312 possible dissipation pathways, such as NI leaching, in the field practice (Kelliher et al.
313 2014). The third reason is that the substrate affinity or capacity to access substrates
314 elicited by varying microbial activities may change along with the changes in

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315 temperature (Wallenstein and Hall 2012). These findings demonstrated that NH_4^+ -
316 based fertilizers with added NI can preserve NH_4^+ -N in soils, but the efficiency of
317 NIs drastically reduces with rising temperature. Consequently, the possible effect on
318 the environment and crops treated with fertilizers coupled with NI application can be
319 dramatically diminished if the temperature is close to or beyond 30 °C. Therefore, soil
320 temperature should be considered to efficiently use NIs.

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322 **Effects of NIs on nitrification rate**

323 The n_{gross} rates in two pasture soils with ACI treatment ranged from 7.12 mg N kg⁻¹
324 day⁻¹ to 15.2 mg N kg⁻¹ day⁻¹. Booth et al. (2005) assembled n_{gross} data from 100
325 studies conducted in grassland, forest, shrubland, and agricultural systems. Most of
326 the rates were lower than 10 mg N kg⁻¹ day⁻¹. The high nitrification rates in the two
327 ACI-treated pasture soils will increase the risk of NO_3^- loss via leaching or
328 denitrification or N_2O emission in field practice. Applying NIs effectively decreased
329 the n_{gross} and n_{net} rates (Fig. 3). The decrease in NO_3^- accumulation approximated 10.8%
330 to 56.2% in the two soils (Figs. 1 and 2). Our results demonstrated that NIs exhibited
331 efficacy in inhibiting both n_{gross} and n_{net} rates in the soils used under laboratory
332 conditions, and this finding may be useful in reducing NO_3^- -N loss via denitrification
333 and leaching processes. Consistently, Di and Cameron (2016) reviewed NIs, including
334 DCD and DMPP, in decreasing N_2O emission and NO_3^- leaching in pasture soils.
335 Their study revealed that NO_3^- leaching from grazed pastures can be decreased by 30%
336 to 50%.

337 NH_4^+ -N concentrations decreased, whereas NO_3^- -N concentrations increased as
338 incubation progressed. NI application slowed down the variations of both NH_4^+ -N
339 and NO_3^- -N (Figs. 1 and 2). However, the observed increases in NO_3^- -N

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340 concentrations were much faster than the declines in $\text{NH}_4^+\text{-N}$ concentrations. These
341 results occurred possibly because $\text{NO}_3^-\text{-N}$ in the two pasture soils was probably not
342 only produced through autotrophic nitrification ($\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$) but also by
343 other N transformation processes, such as heterotrophic nitrification. Previous studies
344 showed that heterotrophic nitrification is the dominating NO_3^- production process in
345 acid soils with high organic C (Zhang et al. 2015a). Therefore, partial inhibition of
346 NIs on NO_3^- production in the two pasture soils may be ascribed to heterotrophic
347 nitrification.

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349 **Effects of NIs on N_2O emission rate**

350 N_2O flux was correlated with the n_{net} rate in soil (Fig. 7), thereby suggesting that NIs
351 can partly inhibit nitrification-related N_2O emission. Moreover, the different NIs
352 reduced N_2O emissions to different extents. DMPP generally reduced N_2O emission
353 more than DCD and 3MP+TZ at 15 °C and 35 °C, whereas DCD performed better
354 than DMPP and 3MP+TZ at 25 °C. Weiske et al. (2001) observed a more significant
355 inhibition in N_2O emissions under field conditions after DMPP application than after
356 DCD application. By contrast, Di and Cameron (2012) cannot find any difference
357 between DCD and DMPP in terms of inhibition of N_2O emission under field
358 conditions. In the present study, the effects of NIs on inhibiting cumulative N_2O
359 emission were lower than that on nitrification rate. This result may be attributed to the
360 high ratio of C and NO_3^- in dairy pasture soil (Table 1); this condition reduced the
361 efficiency of inhibitory effect on N_2O production (Davidson et al. 1986). Another
362 possible explanation is that N_2O release was due to processes, aside from autotrophic
363 nitrification, such as denitrification or heterotrophic nitrification, where NIs caused
364 less effect on N_2O emission. N_2O flux was positively correlated with $\text{NO}_3^-\text{-N}$

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365 concentrations (Fig. 7). This result suggested that NO_3^- , which is a product of
366 nitrification and a substrate for denitrification, was an important indicator for N_2O
367 emission from the studied pasture soils. Furthermore, previous studies have shown
368 that the contribution of heterotrophic nitrification to N_2O emission is much higher
369 than that of autotrophic nitrification in several acidic soils with high soil organic C
370 contents (Liu et al. 2015a; Zhang et al. 2011; Zhang et al. 2015b). Therefore, the
371 importance of heterotrophic nitrification to N_2O emission in tested pasture soils
372 requires further exploration, and this knowledge is valuable for the effective use of
373 NIs in pasture soils with high soil C content.

374 In contrast to the lack of significant difference in n_{gross} rates between 25 °C and
375 35 °C, the average N_2O production rate was much larger at 35 °C than at 25 °C and
376 15 °C regardless of treatment. High N_2O production rate at 35 °C may be ascribed to
377 the increased contribution of denitrification to N_2O emission. Evidence has shown
378 that variation in incubation temperatures may result in changes in soil microbial
379 populations for denitrification (Braker et al. 2010) or differential expression of the
380 enzymes responsible for different $\text{N}_2\text{O}/\text{N}_2$ emissions during denitrification (Saleh-
381 Lakha et al. 2009). According to Phillips et al. (2015), the denitrified N shifts toward
382 N_2O when temperature increases. Therefore, the temperature dependence of N_2O
383 emission is an important environmental factor that will benefit the successful use of
384 NIs in systems with repeated NI applications.

385 386 **Effects of NIs on AOA and AOB abundance**

387 Our study revealed that AOB, but not AOA, significantly decreased after NI
388 application (Figs. 5 and 6). This result is in agreement with the findings of previous
389 studies (Liu et al. 2015b; Shi et al. 2016). The different responses of AOB and AOA

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390 to NI addition may be due to the following reasons. First, nitrification in soils
391 containing high levels of $\text{NH}_4^+\text{-N}$ is mainly followed by the dynamics of AOB
392 abundance rather than AOA abundance (Di et al. 2009). Second, the different
393 compositions of the cell membranes of AOB and AOA (fatty acids as side chains in
394 AOB cell walls versus isoprenoid side chains in the membranes of AOA) influence
395 the cell membrane permeability of NIs (Benckiser et al. 2013; Ruser and Schulz 2015).
396 However, all the three NIs inhibited AOB growth but to different extents. This result
397 indicated that the responses of AOB to different NIs may vary.

398 In the current study, AOA were generally the predominant ammonia oxidizer
399 (Figs. 5 and 6) in the two examined pasture soils with soil pH <6.0. Previous findings
400 suggested that soil pH is a key factor driving the niche partitioning of AOA and AOB
401 (Liu et al. 2015b; Hu et al. 2014; Shi et al. 2016). AOA and AOB populations in the
402 two pasture soils decreased in size with increasing soil temperature from 15 °C to
403 35 °C (Figs. 5 and 6, respectively). Soil bacterial ammonia oxidizer communities
404 were affected by temperature, similar to the results of previous studies (Avrahami et
405 al. 2003; Avrahami et al. 2011; Gubry-Rangin et al. 2017). According to Gubry-
406 Rangin et al. (2017), temperature influences AOA and AOB community compositions
407 in most soils. This effect may be due to the rapid growth of thaumarchaeotal
408 populations at different temperature optima, whereas increasing temperature may
409 increase the death of susceptible community members. The essential differences in
410 temperature-influenced properties of nitrification driven by AOA and AOB support
411 the hypothesis that the biochemical processes associated with NH_3 oxidation in AOA
412 and AOB differ thermodynamically from each other.

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414 **Conclusion**

415 DCD, DMPP, and 3MP+TZ can decrease the n_{net} and n_{gross} rates and N₂O productions
416 from pasture soils but to different extents. NIs reduced nitrification rates by inhibiting
417 AOB growth but not AOA growth. However, the inhibitory effects of NIs on
418 nitrification rates were temperature-dependent, that is, decreasing with increasing
419 temperature. N₂O production in the two pasture soils was only partly inhibited by NIs.
420 Hence, high organic C in two pasture soils may lead to denitrification and
421 heterotrophic nitrification, which can contribute to N₂O production. This aspect
422 requires further study. We also measured AOA and AOB *amoA* gene abundances on
423 the basis of soil DNA. Measuring active communities using soil RNA is highly
424 desirable in future studies.

425
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433
434 **References**

435 AGO (2010) National Greenhouse Account, National Inventory Report 2008, Volume
436 2. Australian Greenhouse Office, Commonwealth of Australia, Canberra.

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51
52
53
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55
56
57
58
59
60
61
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- 437 Akiyama H, Yan X, Yagi K (2010) Evaluation of effectiveness of enhanced-
438 efficiency fertilizers as mitigation options for N₂O and NO emissions from
439 agricultural soils: meta-analysis. *Global Change Biol* 16:1837–1846
- 440 Avrahami S, Bohannan BJA (2007) Response of *Nitrosospira* sp strain AF-Like
441 ammonia oxidizers to changes in temperature, soil moisture content, and
442 fertilizer concentration. *Appl Environ Microbiol* 73: 1166–1173
- 443 Avrahami S, Jia ZJ, Neufeld JD, Murrell JC, Conrad R, Kusel K (2011) Active
444 autotrophic ammonia-oxidizing bacteria in biofilm enrichments from simulated
445 creek ecosystems at two ammonium concentrations respond to temperature
446 manipulation. *Appl Environ Microbiol* 77:7329–7338
- 447 Avrahami S, Liesack W, Conrad R (2003) Effects of temperature and fertilizer on
448 activity and community structure of soil ammonia oxidizers. *Environ Microbiol*
449 5:691–705
- 450 Bauhus J, Meyer AC, Brumme R (1996) Effect of the inhibitors nitrapyrin and
451 sodium chlorate on nitrification and N₂O formation in an acid forest soil. *Biol*
452 *Fert Soils* 22:318–325
- 453 Benckiser G, Christ E, Herbert T, Weiske A, Blome J, Hardt M (2013) The
454 nitrification inhibitor 3,4-dimethylpyrazole-phosphat (DMPP) - quantification
455 and effects on soil metabolism. *Plant Soil* 371:257–266
- 456 Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial
457 ecosystems: a synthetic analysis of literature data. *Ecol Monogr* 75:139–157
- 458 Braker G, Schwarz J, Conrad R (2010) Influence of temperature on the composition
459 and activity of denitrifying soil communities. *FEMS Microbiol Ecol* 73:134–148
- 460 Bremner JM (1997) Sources of nitrous oxide in soils. *Nutr Cycl Agroecosys* 49:7–16
- 461 Davidson EA, Swank WT, Perry TO (1986) Distinguishing between nitrification and

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462 denitrification as sources of gaseous nitrogen production in soil. *Appl Environ*
463 *Microbiol* 52:1280–1286

464 Di HJ, Cameron KC (2004) Effects of temperature and application rate of a
465 nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial
466 biomass in a grazed pasture soil. *Aust J Soil Res* 42:927–932

467 Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009)
468 Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils.
469 *Nat Geosci* 2:621–624

470 Di HJ, Cameron KC (2012) How does the application of different nitrification
471 inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in
472 grazed pastures? *Soil Use Manage* 28:54–61

473 Di HJ, Cameron KC, Podolyan A, Robinson A (2014) Effect of soil moisture status
474 and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier
475 growth and nitrous oxide emissions in a grassland soil. *Soil Biol Biochem* 73:
476 59–68

477 Di HJ, Cameron KC (2016) Inhibition of nitrification to mitigate nitrate leaching and
478 nitrous oxide emissions in grazed grassland: a review. *J Soil Sediment* 16: 1401–
479 1420

480 Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and
481 diversity of ammonia-oxidizing archaea in water columns and sediments of the
482 ocean. *P Natl Acad Sci USA* 102:14683–14688

483 Friedl J, Scheer C, Rowlings DW, Mumford MT, Grace PR (2017) The nitrification
484 inhibitor DMPP (3,4-dimethylpyrazole phosphate) reduces N₂ emissions from
485 intensively managed pastures in subtropical Australia. *Soil Biol Biochem* 108:
486 55–64

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58
59
60
61
62
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64
65
- 487 Gubry-Rangin C, Novotnik B, Mandic-Mulec I, Nicol GW, Prosser JI (2017a)
488 Temperature responses of soil ammonia-oxidising archaea depend on pH. *Soil*
489 *Biol Biochem* 106: 61–68
- 490 Guo YJ, Di HJ, Cameron KC, Li B (2014) Effect of application rate of a nitrification
491 inhibitor, dicyandiamide (DCD), on nitrification rate, and ammonia-oxidizing
492 bacteria and archaea growth in a grazed pasture soil: an incubation study. *J Soil*
493 *Sediment* 14(5):897–903
- 494 Hu XK, Su F, Ju XT, Gao B, Oenema O, Christie P, Huang BX, Jiang RF, Zhang FS
495 (2013) Greenhouse gas emissions from a wheat-maize double cropping system
496 with different nitrogen fertilization regimes. *Environ Pollut* 176:198–207
- 497 Hu HW, Xu ZH, He JZ (2014) Ammonia-oxidizing archaea play a predominant role
498 in acid soil nitrification. In: Sparks DL (ed) *Advances in Agronomy*, Vol 125.
499 Elsevier Science & Technology, pp 261–302
- 500 Hu HW, He JZ (2017) Comammox-a newly discovered nitrification process in the
501 terrestrial nitrogen cycle. *J Soils Sediments* 17: 2709-2717
- 502 IPCC. *Climate change (2007): the physical science basis*. In: Solomon S, Qin D,
503 Manning M (eds). *Contribution of working group I to the fourth assessment*
504 *report of the Intergovernmental Panel on Climate Change*. Cambridge:
505 Cambridge University Press
- 506 Kelliher FM, van Koten C, Kear MJ, Sprosen MS, Ledgard SF, de Klein CAM,
507 Letica SA, Luo J, Rys G (2014) Effect of temperature on dicyandiamide (DCD)
508 longevity in pastoral soils under field conditions. *Agr Ecosyst Environ* 186: 201–
509 204

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61
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63
64
65

510 Kirkham D, Bartholomew WV (1954) Equations for following nutrient
511 transformations in soil, utilizing tracer data: I. Society of America Journal 18:
512 33–34

513 Kumar U, Jain MC, Pathak H, Kumar S, Majumdar D (2000) Nitrous oxide emission
514 from different fertilizers and its mitigation by nitrification inhibitors in irrigated
515 rice. *Biol Fert Soils* 32:474–478

516 Lan T, Han Y, Roelcke M, Nieder R, Cai Z (2013) Effects of the nitrification inhibitor
517 dicyandiamide (DCD) on gross N transformation rates and mitigating N₂O
518 emission in paddy soils. *Soil Biol Biochem* 67: 174–182

519 Liu R, Suter H, Hayden H, He JZ, Chen DL (2015a) Nitrate production is mainly
520 heterotrophic in an acid dairy soil with high organic content in Australia. *Biol*
521 *Fert Soils* 51: 891–896

522 Liu R, Hayden H, Suter H, He J, Chen D (2015b) The effect of nitrification inhibitors
523 in reducing nitrification and the ammonia oxidizer population in three
524 contrasting soils. *J Soil Sediment* 15:1113–1118

525 McCarty GW (1999) Modes of action of nitrification inhibitors. *Biol Fert Soils* 29: 1–
526 9

527 McTaggart IP, Clayton H, Parker J, Swan L, Smith KA (1997) Nitrous oxide
528 emissions from grassland and spring barley, following N fertiliser application
529 with and without nitrification inhibitors. *Biol Fert Soils* 25:261–268

530 Menendez S, Barrena I, Setien I, Gonzalez-Murua C, Maria Estavillo J (2012)
531 Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions
532 under different temperature and moisture conditions. *Soil Biol Biochem* 53: 82–
533 89

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46
47
48
49
50
51
52
53
54
55
56
- 534 Merino P, Menendez S, Pinto M, Gonzalez-Murua C, Estavillo JM (2005) 3,4-
535 dimethylpyrazole phosphate reduces nitrous oxide emissions from grassland after
536 slurry application. *Soil Use Manage* 21:53–57
- 537 Müller C, Stevens RJ, Laughlin RJ (2004) A ¹⁵N tracing model to analyse N
538 transformations in old grassland soil. *Soil Biol Biochem* 36:619–632
- 539 Phillips RL, McMillan AMS, Palmada T, Dando J, Giltrap D (2015) Temperature
540 effects on N₂O and N₂ denitrification end-products for a New Zealand pasture
541 soil. *N Z J Agric Res* 58:89–95
- 542 Puttanna K, Gowda NMN, Rao E (1999) Effect of concentration, temperature,
543 moisture, liming and organic matter on the efficacy of the nitrification inhibitors
544 benzotriazole, o-nitrophenol, m-nitroaniline and dicyandiamide. *Nutr Cycl*
545 *Agroecosys* 54:251–257
- 546 Ruser R, Schulz R (2015) The effect of nitrification inhibitors on the nitrous oxide
547 (N₂O) release from agricultural soils-a review. *J Plant Nutr Soil Sc* 178: 171–188
- 548 Saghir NS, Mulvancy RL, Azam F (1993) Determination of nitrogen by
549 microdiffusion in mason Jars. I. inorganic nitrogen in soil extracts. *Commun Soil*
550 *Sci Plan* 24:1745–1762
- 551 Sahrawat KL (2008) Factors affecting nitrification in soils. *Commun. Soil Sci Plan*
552 39:1436–1446
- 553 Saleh-Lakha S, Shannon KE, Henderson SL, Goyer C, Trevors JT, Zebarth BJ,
554 Burton DL (2009) Effect of pH and temperature on denitrification gene
555 expression and activity in *Pseudomonas mandelii*. *Appl Environ Microbiol* 75:
556 3903–3911

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47
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49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- 557 Sharma SN, Prasad R (1996) Use of nitrification inhibitors (neem and DCD) to
558 increase N efficiency in maize-wheat cropping system. *Fertilizer Research* 44:
559 169–175
- 560 Shi XZ, Hu HW, He JZ, Chen DL, Suter HC (2016) Effects of 3,4-dimethylpyrazole
561 phosphate (DMPP) on nitrification and the abundance and community
562 composition of soil ammonia oxidizers in three land uses. *Biol Fert Soils* 52:
563 927–939
- 564 Weiske A, Benckiser G, Herbert T, Ottow JCG (2001) Influence of the nitrification
565 inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to
566 dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and
567 methane oxidation during 3 years of repeated application in field experiments.
568 *Biol Fert Soils* 34:109–117
- 569 Wallenstein MD, Hall EK (2012) A trait-based framework for predicting when and
570 where microbial adaptation to climate change will affect ecosystem functioning.
571 *Biogeochemistry* 109:35–47
- 572 Zerulla W, Barth T, Dressel J, Erhardt K, von Locquenghien KH, Pasda G, Radle M.,
573 Wissemeier AH (2001) 3,4-Dimethylpyrazole phosphate (DMPP) - a new
574 nitrification inhibitor for agriculture and horticulture - An introduction. *Biol Fert*
575 *Soils* 34:79–84
- 576 Zhang JB, Muller C, Zhu TB, Cheng Y, Cai ZC (2011) Heterotrophic nitrification is
577 the predominant NO_3^- production mechanism in coniferous but not broad-leaf
578 acid forest soil in subtropical China. *Biol Fert Soils* 47:533–542
- 579 Zhang J, Zhu T, Meng T, Zhang Y, Yang J, Yang W, Müller C, Cai Z (2013)
580 Agricultural land use affects nitrate production and conservation in humid
581 subtropical soils in China. *Soil Biol Biochem* 62:107–114

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582 Zhang JB, Muller C, Cai ZC (2015a) Heterotrophic nitrification of organic N and its
583 contribution to nitrous oxide emissions in soils. *Soil Biol Biochem* 84:199–209
584 Zhang JB, Wang J, Zhong WH, Cai ZC (2015b) Organic nitrogen stimulates the
585 heterotrophic nitrification rate in an acidic forest soil. *Soil Biol Biochem* 80:
586 293–295

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Table 1 Soil properties

Soil	Organic matter (%)	Organic C (%)	pH (1:5 water)	CEC (c mol kg ⁻¹)	Clay (<2 µm, %)	Silt (2–60 µm, %)	Sand (60–2000 µm, %)	Soil texture	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
Terang (TR)	7.9	4.60	5.50	7.67	8	64	29	Sandy loam	7.4	16.0
Glenormiston (GN)	10.0	5.90	6.00	24.0	11	52	36	Sandy loam	7.7	44.0

1 **Fig. 1** Dynamics of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ under different temperatures in GN soil during the
2 28-day incubation. Error bars indicate the standard errors of four replicates. GN: soil from
3 Glenormiston, TR: soil from Terang, ACl: $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3$, DMPP: $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3+\text{DMPP}$
4 (DMPP at 0.7% applied $\text{NH}_4^+\text{-N}$), 3MP-TZ: $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3+3\text{MP}+\text{TZ}$ (3MP at 0.33%
5 applied $\text{NH}_4^+\text{-N}+\text{TZ}$ at 0.67% applied $\text{NH}_4^+\text{-N}$), DCD: $\text{NH}_4\text{Cl}+\text{K}^{15}\text{NO}_3+\text{DCD}$ (DCD at 4.5%
6 applied $\text{NH}_4^+\text{-N}$). Numbers 15, 25, and 35 represent the incubation temperatures of 15 °C,
7 25 °C, and 35 °C, respectively
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17 **Fig. 2** Dynamics of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ under different temperatures in TR soil during 28-
18 day incubation. Error bars indicate the standard errors of four replicates. The legend from Fig.
19 1 also applies to this figure.
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25 **Fig. 3** Average net (0–28 day) and n_{gross} rates (0–7 day) from GN and TR soils treated with
26 different NIs. Error bars indicate the standard errors of four replicates. Different letters
27 indicate significant difference among treatments at the same temperature ($P<0.05$).
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33 **Fig. 4** Average daily N_2O production rate from GN and TR soils treated with different NIs.
34 Error bars indicate the standard errors of four replicates. Different letters indicate significant
35 difference among treatments at the same temperature ($P<0.05$).
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41 **Fig. 5** Changes in AOA and AOB *amoA* gene copy numbers in GN soil during the 28-day
42 incubation after treatment with different NIs and temperatures. Error bars indicate the
43 standard errors of four replicates.
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49 **Fig. 6** Changes in AOA and AOB *amoA* gene copy numbers in TR soil during 28-day
50 incubation after treatment with different NIs and temperatures. Error bars indicate the
51 standard errors of four replicates.
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Fig. 7 Relationships between the average daily N₂O production rate and (a) NO₃⁻N and (b) *n*_{net} rates. Dashed curves correspond to 95% confidence interval for linear regression.

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Fig. 1

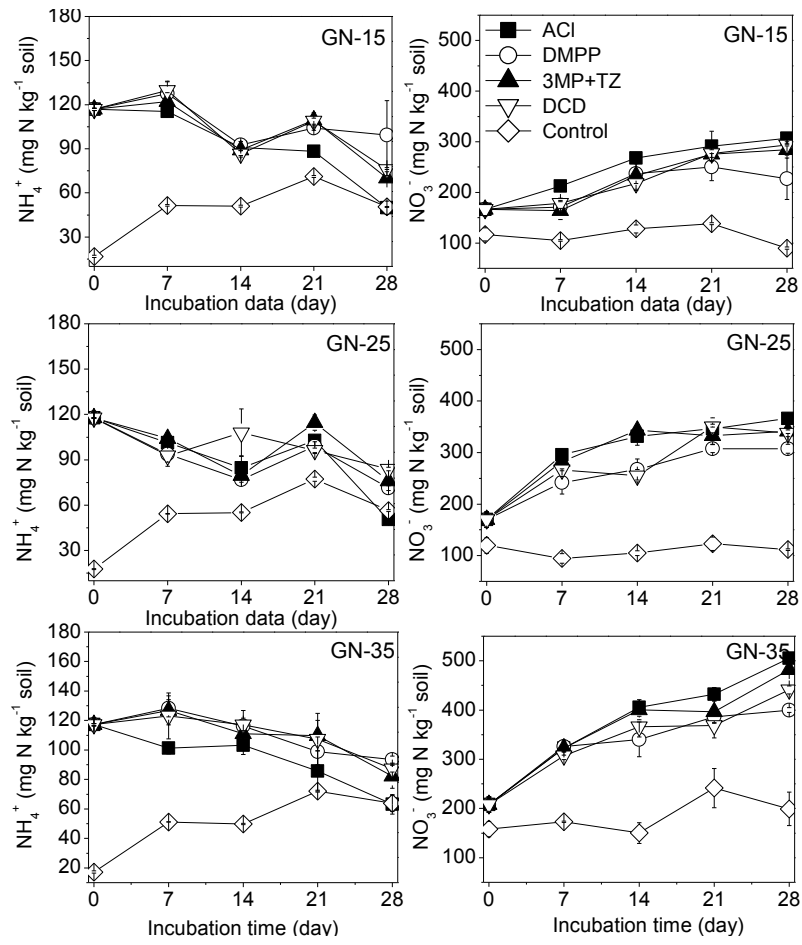


Fig. 2

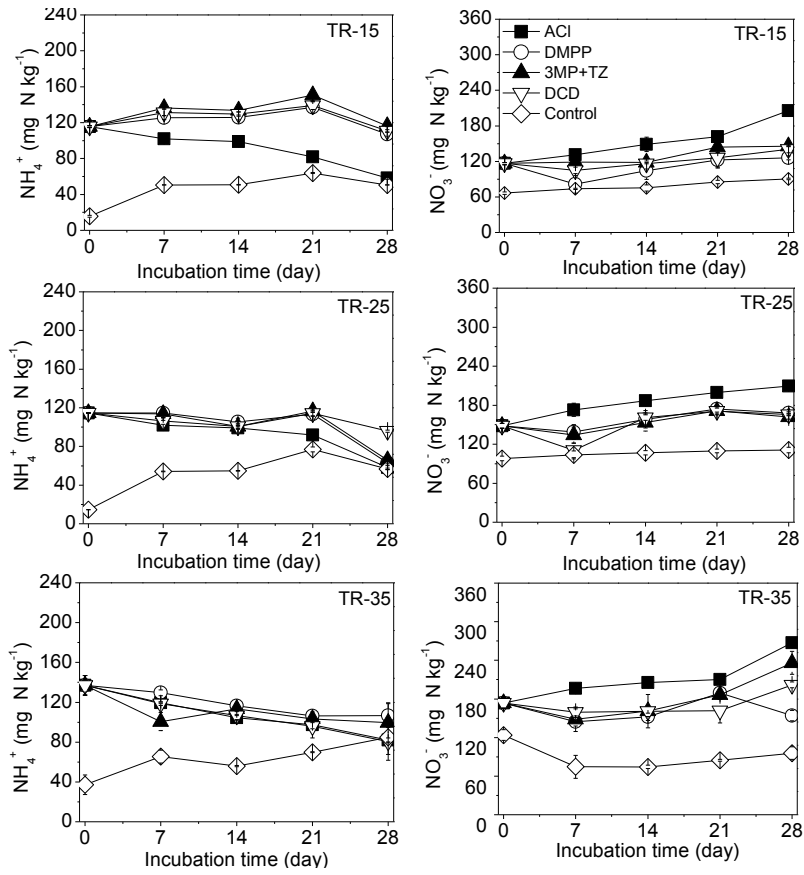


Fig. 3

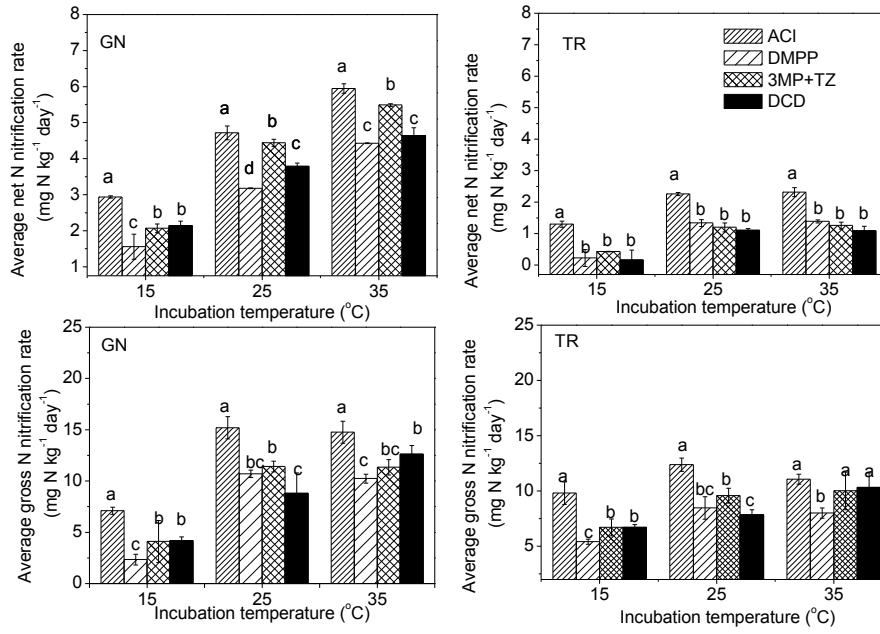


Fig. 4

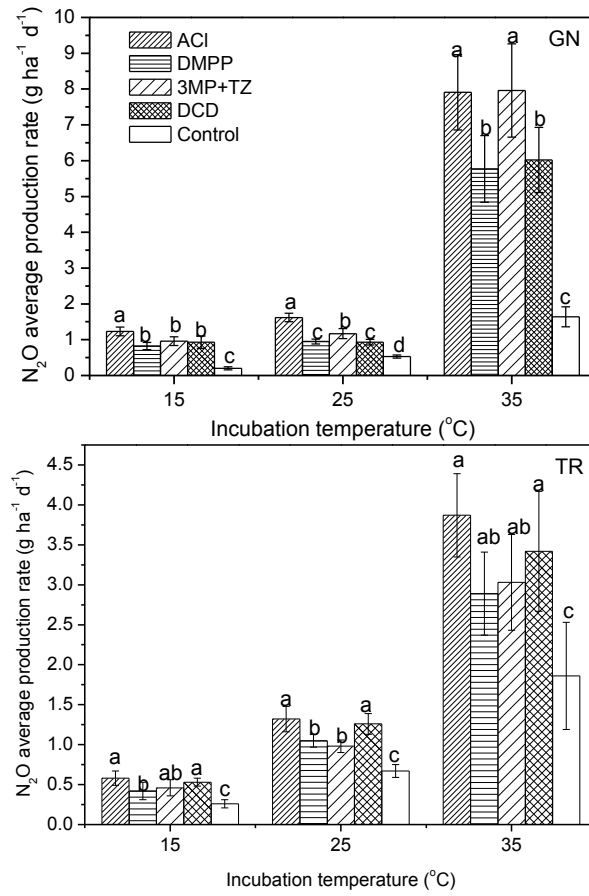


Fig. 5

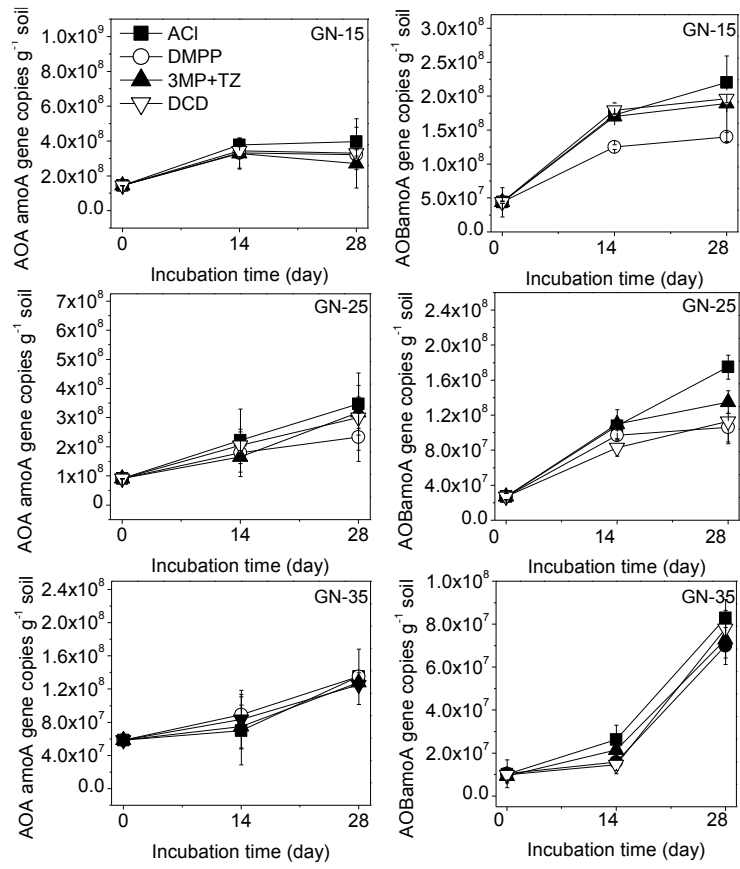


Fig. 6

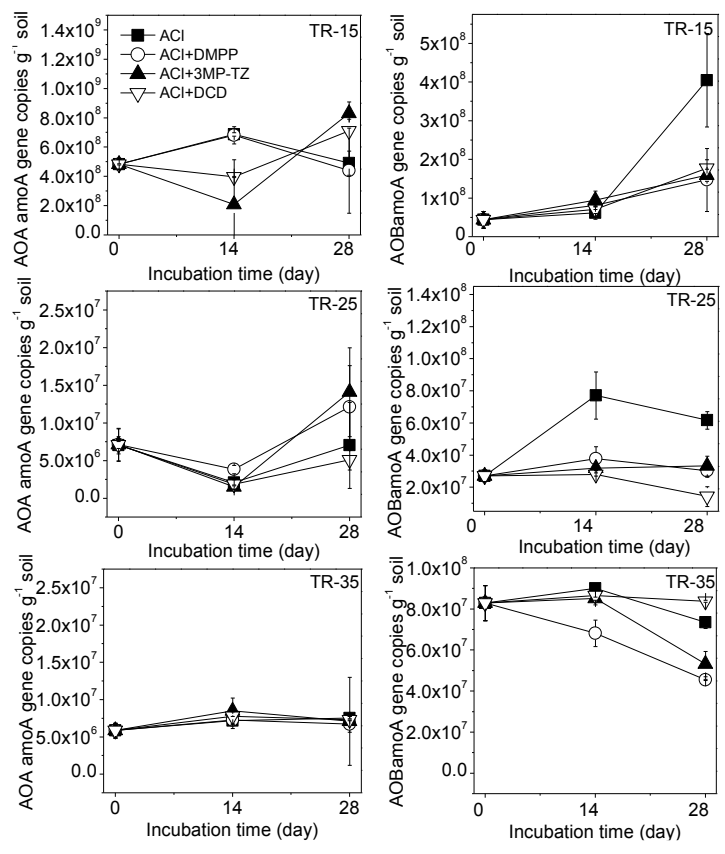
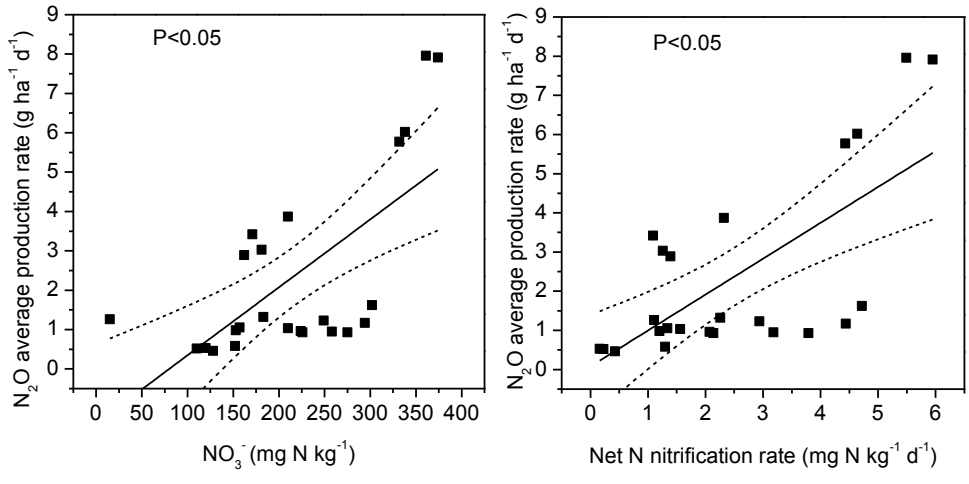
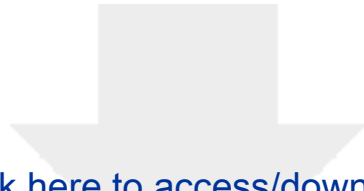


Fig. 7

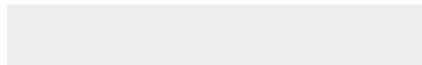


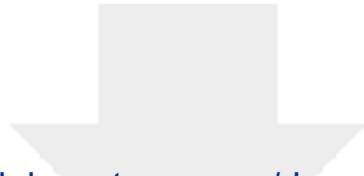


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