1 **Nitrification responses of soil ammonia-oxidizing archaea and bacteria to ammonia**

2 **additions.**

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23   **Abstract**

24   Although ammonia-oxidizing archaea (AOA) and bacteria (AOB) co-exist in most non-acidic

25   agricultural soils, the factors that influence their relative contributions to soil nitrification activity

26   remain unclear. A 2-4 d whole soil microcosm assay was developed, utilizing the aliphatic C8-

27   alkyne, 1-octyne, to inactivate AOB driven nitrification activity without impacting AOA

28   nitrification activity. Responses of AOA and AOB nitrification activities (accumulation of NO_2^-

29    $+NO_3^-$) to different concentrations of extractable ammonium (NH_4^+) were examined in four

30   diverse, paired cropped and non-cropped Oregon soils sampled in summer and winter. Maximum

31   AOA nitrification rates were significantly higher in non-cropped (3.7mg N kg^{-1} soil d^{-1}) than in

32   cropped soils (1.0mg N kg^{-1} soil d^{-1}), and in summer (3.1mg N kg^{-1} soil d^{-1}) compared to winter

33   soils (1.6mg N kg^{-1} soil d^{-1}). The NH_4^+ concentration required to significantly stimulate AOB

34   nitrification activity was significantly higher in cropped soils (67mg N kg^{-1} soil) than in non-

35   cropped soils (12mg N kg^{-1} soil). Maximum AOB activity was significantly higher in cropped

36   (8.6mg N kg^{-1} soil d^{-1}) than in non-cropped soils (2.9mg N kg^{-1} soil d^{-1}), and in summer (7.8mg

37   N kg^{-1} soil d^{-1}) compared to winter soils (3.8mg N kg^{-1} soil d^{-1}). This study has revealed that

38   AOA and AOB nitrification rates respond differently to NH_4^+, that cropping influences their

39   relative contributions to nitrification, and that season of sampling impacts nitrification rates.

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41   Abbreviations: AOA, Ammonia oxidizing archaea; AOB ammonia oxidizing bacteria; SC

42   summer cropped; WC, winter cropped; SNC, summer non-cropped; WNC, winter non-cropped.

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Introduction

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Nitrification is the microbially mediated transformation of ammonium (NH_4^+) to nitrite (NO_2^-)

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51 and subsequently to nitrate (NO_3^-). Ammonia (NH_3) oxidation is the first and rate limiting step in

52 the nitrification process, and is carried out by ammonia-oxidizing archaea (AOA) and bacteria

53 (AOB). Although AOB have been extensively studied for 130+ years, AOA were only

54 discovered recently (Konneke et al., 2005; Treusch et al., 2005). Since the discovery of AOA, it

55 has been revealed that AOA are abundant in soil and frequently outnumber AOB (Alves et al.,

56 2013; Leininger et al., 2006; Nicol et al., 2008; Wessen et al., 2011). Despite AOA abundance, it

57 remains unclear what factors control the contributions of AOA to soil nitrification. There is

58 evidence from marine systems to suggest that AOA and AOB exhibit a niche separation based on

59 their respective affinities for NH_3, and that AOA are dominant under low NH_3 conditions

60 (Martens-Habbena et al., 2009). In soil systems there is evidence that pH separates AOA and

61 AOB contributions, with AOA dominating at low pH, which may be linked to NH_3 availability

62 (Gubry-Rangin et al., 2010; Lehtovirta-Morley et al., 2011; Nicol et al., 2008). In most soils

63 AOA and AOB coexist, yet it is not known what controls their relative activities. Recently

64 Taylor et al. (2013) described a procedure that discriminates between AOA and AOB activities

65 and obtained evidence for seasonal and cropping effects on the contributions of AOA and AOB

66 to nitrification in soil slurries.

67 The aim of this study was to extend the above work and examine the response of both total and

68 relative contributions of AOA and AOB nitrification activities to incremental increases in NH_4^+

69 concentrations in cropped and non-cropped soils sampled in summer and winter. Gaseous

70 additions of 1-octyne and NH_3 to the soils allowed these experiments to be performed in

71 unsaturated whole soils. Previous studies have used gaseous NH_3 additions to examine

72 nitrification in soil at various unsaturated water contents (Murphy et al., 1999, 1997; Stark and

73 Firestone, 1995; Taylor et al., 2013). We hypothesized: i) that AOA would respond to lower

74 levels of NH_4^+ than AOB, given that AOA have been shown to have a much higher affinity for

75 NH_4^+ (Martens-Habbena et al., 2009); ii) that AOA activity would dominate in non-cropped

76 soils, as they do not receive NH_4^+ additions, and AOB would dominate cropped soils, as they

77 regularly receive NH_4^+ fertilization (Taylor et al., 2010, 2013); and iii) that there would be

78 greater nitrification activity in summer soils, compared to winter soils for both AOA and AOB

79 (Taylor et al., 2010).

80 **Materials and methods**

81 *Soil sampling*

82 Cropped and non-cropped soils were sampled from four locations in Oregon. Samples were

83 collected from: i) Columbia Basin Agricultural Research Center, Pendleton; ii) Central Oregon

84 Agricultural Research Center, Madras; iii) Klamath Basin Research & Extension Center,

85 Klamath Falls; iv) Hyslop Crop Science Field Research Laboratory, Corvallis. From each

86 location three samples were collected from cropped and adjacent non-cropped surface soils (0-20

87 cm). Samples were collected in the summer of 2012 and the winter of 2013, and stored at 4ºC

88 until used.

89 *Site Description*

90 The Columbia Basin Agricultural Research Center, is located in northeast Oregon

91 (45°43'9.92"N, 118°37'37.24"W). It receives a mean of 360 mm of precipitation annually and

92 has a mean annual temperature of 11ºC. The soil at this site is classified as a coarse-silty, mixed,

93 superactive, mesic Typic Haploxerolls (Soil Survey Staff, 2014). The cropped soil was in a

94 wheat-fallow cropping rotation and the adjacent non-cropped soil component represents a

95 remnant grassland that has never been cultivated. The Central Oregon Agricultural Research

96 Center is located in central eastern Oregon (44°40'52.38"N, 121° 8'56.14"W). It receives a mean

97 of 250 mm of precipitation annually and has a mean annual temperature of 9ºC. The soil at this

98 site is classified as fine-loamy, mixed, superactive, mesic Aridic Argixerolls (Soil Survey Staff,

99 2014). The cropped soil is cultivated for root crop seed production and the non-cropped soil

100 occurs under sage brush. Klamath Basin Research & Extension Center is located in south central

101 Oregon. (42° 9'57.09"N, 121°45'27.53"W). It receives a mean of 300 mm of precipitation

102 annually and has a mean annual temperature of 8ºC. The soil on this site is classified as sandy,

103 mixed, mesic Typic Durixerepts (Soil Survey Staff, 2014). Cropped soils are under a wheat

104 rotation and the adjacent non-cropped soil occurs under a pine woodlot, which has never been

105 cultivated. Hyslop Crop Science Field Research Laboratory in Corvallis is located in western

106 Oregon (44°38'1.64"N, 123°11'38.99"W). It receives a mean of 1140 mm of annual rainfall and

107   has a mean annual temperature of 11ºC. Soil at this site is classified as fine-silty, mixed,

108   superactive, mesic Aquultic Argixerolls (Soil Survey Staff, 2014). Cropped soils are under a

109   wheat-fallow rotation and non-cropped soils were removed from cultivation and seeded over

110   with mixed grass species ~20 years ago. Soil properties are described in Table 1.

Determination of NO_3^-, NO_2^- and NH_4^+

112   Net nitrification activity was determined by quantifying total NO_3^- and NO_2^- accumulation. Soil

113   (2.5 g) was extracted with 15 ml distilled water for 15 min. Samples were centrifuged, and the

114   supernatants analyzed colorimetrically using the method described by Miranda et al. (2001).

115   Extractable NH_4^+ was determined after extracting 2.5 g soils with 15 ml 2 M KCl for 1 h using

116   the method described in Mulvaney (1996).

Whole soil incubations to determine net nitrification activities

118   Prior to incubations the gravimetric water content of soil samples was determined. The three

119   field samples of cropped or non-cropped soil from each location were composited and

120   homogenized prior to incubation. Soils (10 g) were added to 125-ml Wheaton bottles and wet to

121   field capacity and allowed to pre-incubate for 24 h at room temperature (23ºC). Pre-incubation

122   minimized the influence of storage at 4ºC and allowed the added water to equilibrate with the

123   soil prior to substrate and inhibitor addition. Bottles were capped and sealed with n-butyl

124   stoppers. Anhydrous NH_3 was added in amounts sufficient to achieve approximately 14, 28, 70,

125   and 140 mg NH_4^+-N kg^{-1} dry soil. KCl-extractable NH_4^+ concentrations were measured in soil

126   samples recovered from bottles treated with acetylene, to obtain an accurate measurement of the

127   final NH_4^+ concentrations achieved in the soils. Acetylene was prepared by a 10-fold dilution,

128   then adding 300 µl of the dilution to the 125-ml bottles to give a final aqueous concentration of 6

129   µM (0.02 % v/v). A stock preparation of the AOB inhibitor, 1-octyne, was prepared and added to

130   bottles as described by Taylor et al. (2013). Briefly, several glass beads were added to a 125-ml

131   screw cap media bottle fitted with an n-butyl rubber stopper, 40 µl liquid octyne was added, and

132   the bottle over pressured with 100 ml air. The bottles were shaken vigorously, and aliquots of the

133   headspace sampled with a gas tight syringe and 2.7 ml octyne gas was added to bottles to give an

134   aqueous solution concentration of ~4 µM (1.9% v/v). To achieve measureable net nitrification

135   activity, summer soils were incubated and sampled at 2 d; winter soils were incubated and

136   sampled at 2 and 4 d. After each sampling the bottles were left open for 1 h to release the

137   acetylene and octyne, whereupon the bottles were resealed and fresh octyne and acetylene added

138   to achieve the initial concentrations. Three replications were used for each treatment. Significant

139    $NO_2^- + NO_3^-$ accumulation did not occur in the acetylene controls, suggesting that all nitrification

140   activity was due to lithotrophic NH_3 oxidation. Total net nitrification rates were based on the

141   accumulation of $NO_3^- + NO_2^-$ in the absence of gaseous inhibitors. Net nitrification in the

142   presence of 1-octyne (i.e., octyne resistant) was attributed to AOA activity, with AOB activity

143   calculated by difference between the total and AOA nitrification rates (i.e., octyne-sensitive).

Determination of Net N Mineralization rates

145   Net N mineralization was determined with whole soil incubations of 28 d duration. Gravimetric

146   water content was determined, and 40 g of soil was added to 125-ml bottles. Water content was

147   adjusted to field capacity, and soils incubated at 25ºC in the presence and absence of 6 μM_{aq}

148   acetylene. The accumulation of $NO_3^- + NO_2^-$ and NH_4^+ were measured every 7 d. Rates of

149   mineralization were calculated as the accumulation of NH_4^+ in the presence of acetylene from 0-

150   7d.

Determination of soil solution NH_4^+

152   Determination of soil solution NH_4^+ was carried out using a method adapted from McInnes et al.

153   (1994) and Taylor and Bottomley (2006). Dry Whatman #1 filter papers (2x2 cm) were weighed,

154   placed between two other pieces of filter paper, and completely covered by 7.5 g soil. The soil

155   was then wet to field capacity and anhydrous NH_3 additions were made to provide quantities of

156   NH_4^+ ranging between 14, 28, 70, and 140 mg NH_4^+-N kg^{-1} dry soil. Acetylene was added to

157   inhibit all nitrification activity and soils were incubated at 4ºC for 24 hrs. The filter paper was

158   weighed to determine water content, NH_4^+ on the paper was extracted in 2 M KCl, and the

159   concentration of NH_4^+ in solution determined. A portion of the soil was extracted with 2 M KCl

160   and extractable NH_4^+ determined.

Statistics

162   Significant differences in the accumulation of $NO_3^- + NO_2^-$ at different NH_4^+ concentrations were

163   determined using an analysis of variance with Tukey-Kramer adjustment for all pairwise

164   comparisons (Supplemental Fig. 1-4). From these data, three parameters related to total, AOA,

165   and AOB nitrification activity were determined: i) the minimum concentration of NH_4^+ needed to

166   stimulate nitrification activity was chosen as the lowest NH_4^+ that stimulated net nitrification

167   activity above that observed without added NH_4^+; ii) the maximum rate of net nitrification

168   activity was the highest rate of observed net nitrification; and iii) the concentration of NH_4^+

169   required to saturate nitrification activity was selected as the concentrations after which there was

170   no significant stimulation of nitrification activity (Fig. 1). Differences in rates of nitrification,

171   and NH_4^+ concentrations between cropped/non-cropped and summer/winter and fraction of

172   octyne-resistant activity were determined using a two-way analysis of variance, with the four

173   sites as the level of replication. Analysis was performed using Statgraphics X64 software

174   (Statpoint Technologies, Warrenton, VA, USA).

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188   **Results**

189   Figure 1 demonstrates the total, AOA and AOB nitrification responses in on pair of

190   representative paired cropped and non-cropped soil. These nitrification response curves were

191   generated at all locations, for cropped and non-cropped in both summer and winter.

192   *Total net nitrification activity*

193   There were no significant differences in background rates of nitrification by season or cropping

194   treatment (Table 2). The minimum NH_4^+ concentration required to significantly stimulate total

195   nitrification above background in winter cropped (referred to as WC) varied about four-fold

196   among the soils (15-67 mg NH_4^+-N kg^{-1} soil, Supplemental Fig.1), whereas total nitrification

197   activity was only stimulated in one of four winter non-cropped (referred to as WNC) by NH_4^+

198   additions. In summer cropped (referred to as SC), nitrification activity was significantly

199   stimulated by NH_4^+ concentrations that were higher than needed for WC and varied more than

200   six-fold (22-145 mg NH_4^+-N kg^{-1} soil, Supplemental Fig. 2). In summer non-cropped (referred to

201   as SNC), total nitrification activity was stimulated by NH_4^+ concentrations that were lower than

202   needed for SC (14-29 mg NH_4^+-N kg^{-1} soil, Supplemental Fig. 2).

203   The concentration of NH_4^+ needed to saturate total nitrification activity was significantly higher

204   in cropped soils (127±96 mg NH_4^+-N kg^{-1} soil) compared to non-cropped soils (28±24 mg NH_4^+-

205   N kg^{-1} soil; p=0.01). (Supplemental Fig. 1-4). The mean maximum nitrification activity in

206   summer soils (8.5±5 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}) were nearly twice that of winter soils

207   (4.9±2.3 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}; p=0.04). Maximum activity in SC soils was achieved by

208   NH_4^+ concentrations with a mean of 115±23 NH_4^+-N kg^{-1} soil and in two cases could not be

209   saturated even at the highest NH_4^+ concentrations (119 and 146 mg NH_4^+ -N kg^{-1} soil). Maximum

210   nitrification activity in SNC soils were achieved by NH_4^+ concentrations that were substantially

211   lower than SC (28±18 mg NH_4^+-N kg^{-1} soil; p=0.01).

212   *Net AOA nitrification activity*

213   Background AOA activity was detected in five of eight non-cropped soils (two of four WNC and

214   three of four SNC) ranging from 0.69-1.9 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}. Background AOA

215   activity was detected in two of eight cropped soils, (two of four SC) with rates ranging from

216   0.84-1.4 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}. There were no significant differences in background

217   AOA nitrification activity between seasons or treatments.

218   The addition of NH_4^+ stimulated AOA activity in non-cropped soil, while additional NH_4^+ did not

219   stimulate AOA nitrification activity in cropped soils, implying that in cropped soils, AOA

220   activity was saturated by background NH_4^+ concentrations (4.7±3.7 mg NH_4^+-N kg^{-1} soil). The

221   minimum NH_4^+ concentration required to stimulate AOA activity in non-cropped soils (16±13

222   mg NH_4^+-N kg^{-1} soil) was significantly higher than the background NH_4^+ concentrations in

223   cropped soils (p=0.015) (Fig. 2). The concentration of NH_4^+ required to stimulate AOA activity

224   was also significantly higher in summer soils (15±12 mg NH_4^+-N kg^{-1} soil) than in winter soils

225   (5.3±5 mg N kg^{-1} soil; p=0.02) (Fig. 2). Ammonium-stimulated AOA nitrification activity was

226   significantly higher in non-cropped soils (2.9±1.3 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}) compared to

227   cropped (0.6±.4 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}; p=0.0001) soils, and was higher in summer

228   (2.2±1.8 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}) than in winter (1.2±1 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1};

229   p=0.03) soils. Ammonium-stimulated rates in non-cropped soils were compared to background

230   rates in cropped soils, as there was no additional stimulation of AOA nitrification activity by

231   NH_4^+ additions in cropped soils.

232   Maximum AOA nitrification activity was significantly higher in non-cropped (3.7±2.3 mg NO_2^-

233   +NO_3^--N kg^{-1} soil d^{-1}) than in cropped soils (0.9+0.5 mg NO_2^- +NO_3^--N kg^{-1} soil d^{-1}) (p= 0.004)

234   (Fig 3). The mean level of NH_4^+ required to saturate AOA nitrification activity was significantly

235   higher in non-cropped (21±17 mg NH_4^+-N kg^{-1} soil) soils compared to cropped soils (4.5±3.8 mg

236   NH_4^+-N kg^{-1} soil; p=0.009) (Fig 3).

237   ***Fraction of AOA/total nitrification activity***

238   The fraction of AOA activity was significantly greater in SNC (73%±9) than in SC (24%±20)

239   across all NH_4^+ concentrations (p<0.0001). The fraction of AOA activity was also significantly

240   greater in WC (54%±30) than in WNC (16%±8) (p<0.0001). The fraction of octyne resistant

241   nitrification activity in SNC was also significantly greater than in WNC soils (p=0.0002), but did

242   not differ between SC and WC (p=0.23). There was a significant interaction (p=0.04) between

243   cropped/non-cropped and season, so soils were separated for analysis.

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Net AOB nitrification activity

AOB nitrification rates were calculated as the difference between total and AOA nitrification rates. Background AOB activity was detected in only three of eight winter soils (0.5 -1.9 mg $NO_3^-+NO_2^-$ -N kg^{-1} soil d^{-1}), and undetected in summer soils.

The NH_4^+ concentration required to significantly stimulate AOB activity above background was significantly higher in cropped (67 ± 49 mg NH_4^+-N kg^{-1} soil) than in non-cropped (12 ± 10 mg NH_4^+-N kg^{-1} soil) soils (p=0.004) (Fig. 2). AOB activity was stimulated by NH_4^+ additions in all cropped soils, while it was only stimulated two of eight non-cropped soils. When there was no stimulation of AOB nitrification activity, the background KCl extractable NH_4^+ was considered to be the saturating concentration of NH_4^+. There was no effect of season on the concentration of NH_4^+ required to stimulate AOB activity.

The concentration of NH_4^+ required to support the maximum rate of AOB nitrification activity was significantly higher in cropped (116 ± 31 mg NH_4^+-N kg^{-1}) than in non-cropped (30 ± 47 mg NH_4^+-N kg^{-1}) soils (p=0.0036) (Fig. 3). Mean maximum AOB activity was significantly higher in cropped (8.6 ± 6.0 mg $NO_3^- + NO_2^-$-N kg^{-1} soil d^{-1}) than in non-cropped (2.9 ± 1.9 mg $NO_3^- + NO_2^-$-N kg^{-1} soil d^{-1}) soils (p=0.009) (Fig. 3).

Discussion

In this study we have built upon earlier work that showed that the linear C8 alkyne, 1-octyne, selectively and irreversibly inactivates NH_3 oxidation by AOB at very low concentrations (1 μM_{aq}), but does not inhibit AOA activity unless used at 10 to 20-fold higher concentrations (Taylor et al., 2013). Using this method, we examined the influence of season, cropping, and NH_4^+ additions on short-term (≤4 d) rates of AOA (octyne-resistant) and AOB (octyne-sensitive) nitrification, in adjacent cropped and non-cropped soils from four of the major agricultural production regions of Oregon. As mentioned in the introduction, although several studies have been reported in the literature which show that NH_3/NH_4^+ availability, cropping practice, and season are major factors influencing the relative sizes of AOA and AOB populations in soil, there has been little work to compare the relative nitrifying activities of AOA and AOB in soil in response to these different cropping and seasonal soil conditions (Taylor et al., 2012).

In this study the most important factor influencing the relative magnitudes of AOA and AOB nitrification activities was whether the soils were cropped or non-cropped. The maximum AOA rates of nitrification in cropped soils were generally lower than non-cropped soils. For example, SC soils had a mean AOA rate of 1.3±0.7 versus 4.8±2.4 mg $NO_3^- + NO_2^-$ -N kg^{-1} soil d^{-1} in SNC soils. In addition, the AOA rates in cropped soils were not significantly stimulated by additions of NH_4^+, whereas AOA activity was stimulated by NH_4^+ additions in all SNC, suggesting that AOA activity was NH_4^+ limited in the latter soils. Because non-cropped soils had no history of either cultivation or N fertilization, NH_4^+ limitation of AOA activity presumably reflects the fact that the indigenous pool of mineralizable N was insufficient to meet the AOA nitrifying potential at the time of sampling. Furthermore, because the maximum AOA rates were two to four-fold higher in SNC than WNC, the data confirm that the potentially active AOA population was larger in summer than winter. Research findings have been mixed on whether nitrification activity by soil AOA depends upon exogenous additions of NH_4^+. For example, several studies have shown that soil AOA will proliferate and/or incorporate $^{13}CO_2$ into thaumarchaeal DNA when N mineralization is the sole source of NH_4^+ (Jia and Conrad, 2009; Zhang et al., 2010). This result might be expected if soil AOA possess a high affinity for NH_4^+ as shown in the marine thaumarcheon, *N. maritimus* (Martens-Habbena et al., 2009). Other soil studies have shown, however, that AOA population growth can be stimulated above background by additions

305   of low concentrations of NH_4^+ in the order of 14-28 mg NH_4^+-N kg^{-1} soil; implying that AOA are

306   NH_4^+ limited under some soil conditions, and/or that some AOA soil populations do not have

307   exceptionally high affinity for NH_4^+/NH_3 (Taylor et al., 2013; Verhamme et al., 2011). Clearly,

308   our data illustrate that the NH_4^+ concentration required to support maximum activity of AOA

309   varies among soils and that season of sampling might also be influential.

310   In contrast to AOA activity, AOB nitrification rates were stimulated by NH_4^+ additions to higher

311   maximum activities in cropped soils than in non-cropped soils, suggesting that cropped soils

312   contain higher population densities of AOB than non-cropped soils. This is not too surprising

313   since the SC soils were sampled from under crops several weeks after spring N fertilization. In

314   SC, the rates of AOB nitrification were significantly stimulated above background by a mean of

315   95.9 ± 55.4 mg NH_4^+-N kg^{-1} soil, whereas in SNC, AOB activities were significantly stimulated

316   above background by lower concentrations of NH_4^+ (22.2 ± 13.7 mg NH_4^+-N kg^{-1} soil). This

317   observation indicates that the active AOB populations in non-cropped soils might have a higher

318   affinity for NH_4^+ than the AOB active in cropped soils. Evidence has been obtained from pure

319   culture studies that the K_s for NH_4^+/NH_3 varies among different members of the soil dominant

320   *Nitrosospira* lineage (Bollmann et al., 2005; Taylor and Bottomley, 2006), and also that

321   sensitivity to high NH_4^+ concentrations differs among subgroups of *Nitrosospira* (Webster et al.,

322   2005). Although we did not compare AOB community composition between cropped and non-

323   cropped soil, AOB population composition has been shown to differ between soils that are N

324   fertilized versus those not fertilized with N and that AOB abundance increases in N fertilized

325   soils (Di et al., 2009; Prosser and Nicol, 2012; Taylor et al., 2010 Zeglin et al., 2011). In SC

326   soils, the AOA fraction of total nitrification was highest at NH_4^+ concentrations $\leq$70 mg-N kg^{-1}

327   soil, and the increase in the fraction of AOB nitrification at higher NH_4^+ concentrations is most

328   readily explained by AOB populations that develop greater NH_3 oxidizing capacity albeit with

329   lower affinity for NH_4^+/NH_3. We also noted that whereas the AOB activity of WC soils saturated

330   at ~70 mg NH_4^+-N kg^{-1} soil, it could not be saturated in two of the SC soils. Again, this result

331   suggests that the AOB populations responsive to NH_4^+ in SC soils possessed different kinetic

332   properties of NH_3 oxidation than those potentially active in WC soils. The difficulty in saturating

333   nitrification in some SC might be due to the fact that most of the added NH_4^+ was bound to soil

334   exchange sites and soil solution NH_4^+ concentrations did not rise >2mM (Supplemental Fig. 5);

335   K_s NH_4^+ values of some AOB fall in the range of 1-2 mM at circumneutral pH (Hyman and

336   Wood, 1985; Suwa et al., 1994; Suzuki et al., 1974).

337   Lower AOA nitrification activity in cropped soils compared to non-cropped soils may infer that

338   long-term N fertilization negatively impacts AOA populations. Evidence from enrichment and

339   pure culture studies has shown that some AOA are sensitive to moderate concentrations of NH_4^+

340   > 2-3 mM (French et al., 2012; Hatzenpichler, 2012; Konneke et al., 2005). In our study,

341   although nitrification by AOA saturated at low NH_4^+, this activity was not reduced by adding

342   NH_4^+ concentrations realistic of fertilizer N applications. This lack of sensitivity to NH_4^+ can be

343   explained by NH_4^+ concentrations in soil solution, which did not exceed 2 mM even at the

344   highest NH_4^+ concentrations applied (Supplemental Fig. 5), which is a value typically used to

345   culture AOA in the laboratory (Hatzenpichler, 2012; Martens-Habbena et al., 2009; Tourna et al.,

346   2011).

347   Evidence was obtained in this study that season of sampling significantly influenced AOB

348   maximum nitrification rates, and weakly influenced maximum AOA rates (p=0.07). Other

349   studies have shown that season influences AOA and AOB *amoA* gene abundances, and

350   nitrification potential rates fluctuated throughout the year (O'Sullivan et al., 2013; Taylor et al.,

351   2012). In our study, the soil incubations were conducted at 25℃ regardless of season of

352   sampling, yet some studies indicate that soil AOA may show preference for either higher or

353   lower temperatures than 25°C. For example, *N. viennensis* is a soil AOA isolate that exhibits

354   maximum nitrification activity at >35℃ (Tourna et al., 2011), and another study demonstrated

355   that AOA community composition shifted when soil was incubated at 30℃ with little discernible

356   change occurring at incubations ≤25°C (Tourna et al., 2008). In contrast, Alves et al. (2013)

357   showed that the AOA composition of Arctic soil enrichment cultures shifted in response to

358   incubation at 4℃ versus 20℃, and nitrification activity did not persist in enrichments made at

359   28℃ suggesting that differences in temperatures between 4 ℃ and 20°C might be sufficient to

360   influence AOA community composition and their nitrification activity.

361   Previous research has examined the potential of acetylenic compounds to inhibit nitrification in

362   soils. For example, McCarty and Bremner (1986) demonstrated that a wide range of acetylenic

363   compounds inhibit nitrification to varying degrees, and that 1-octyne inhibited 49-77% of

364   nitrification activity in 7-d incubations of three Iowa soils. Our study raises the possibility that

365   selective inhibitors could be employed to reduce the rate of nitrification as a technique in

366   ammoniacal N management. Our data demonstrates that nitrification activity of AOA respond

367   generally to lower NH_4^+ concentrations than AOB, and express lower maximum nitrification

368   rates than AOB in cropped soils. Placing this into a cropping perspective, two of the largest

369   acreage field crops produced in Oregon are grass seed and winter wheat with recommended

370   fertilizer N rates of 106 and 185 kg N ha^{-1}, respectively (Gardner et al., 2000; Petrie et al., 2006).

371   Our study demonstrated that these rates of fertilization were often sufficient to saturate total

372   nitrification activity, and we calculated that under ideal conditions, AOB activity could nitrify all

373   NH_4^+-N applied to grass seed and wheat in 12-22 d, while AOA activity would take 88-154 d to

374   nitrify the same quantity of NH_4^+. The data collected in this study suggest that if a suitable

375   inhibitor for field use could be found, selective inhibition of AOB activity might be a simple N

376   management strategy to reduce N loss from some cropping systems.

377   **Acknowledgements**

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Figure Captions

Figure 1: Rates of Total, AOA and AOB nitrification rates in soil. Dark circles represent total nitrification activity, open circles represent AOA nitrification activity, and dark triangles represent mean AOB activity, calculated as the difference between total and AOA activity. † Represents the minimum concentration required to significantly stimulate nitrification activity, determined using an ANOVA with Tukeys HSD for all pairwise comparisons. ‡ Represents the maximum observed mean nitrification activity. § Represents the minimum level of NH_4^+ required to saturate nitrification activity, determined using an ANOVA with Tukeys HSD for all pairwise comparisons. Error bars represent the standard deviation (n=3)

Figure 2: Minimum concentration of NH_4^+ required to stimulate nitrification activity. Dark bars represent the concentration of NH_4^+ required to stimulate AOA activity, and grey bars represent the concentration of NH_4^+ required to stimulate AOB activity. Error bars represent the standard deviation (n=4)

Figure 3: Maximum nitrification activity. Dark bar represent maximum AOA nitrification activity, and grey bars represent AOB nitrification activity. Error bars represent the standard deviation (n=4)

Supplemental Figure 1: Rates of total nitrification activity of soils sampled in winter 2013. Values with different letters are significantly different as determined with an ANOVA and Tukeys HSD test (p-value ≤ 0.05). Closed circles represent cropped soils, open circles represent non-cropped soils and error bars represent standard deviation (n=3).

Supplemental Figure 2: Rates of total nitrification activity of soils sampled in summer 2012. Values with different letters are significantly different as determined with an ANOVA and Tukeys HSD test (p-value ≤0.05). Closed circles represent cropped soils, open circles represent non-cropped soils and error bars represent standard deviation (n=3).

Supplemental Figure 3: Octyne resistant nitrification activity of soils sampled in winter 2013. Values with different letters are significantly different as determined with an ANOVA and Tukeys HSD test (p-value ≤0.05). Closed circles represent cropped soils, open circles represent non-cropped soils and error bars represent standard deviation (n=3).

540   Supplemental Figure 4: Octyne resistant nitrification activity of soils sampled in summer 2012.

541   Values with different letters are significantly different as determined with an ANOVA and

542   Tukeys HSD test (p-value ≤ 0.05). Closed circles represent cropped soils, open circles represent

543   non-cropped soils and error bars represent standard deviation (n=3)

544   Supplemental Figure 5: The fraction of total nitrification activity that is resistant to 1-octyne.

545   Panels A-D represent soils sampled in winter, and E-F represent soils sampled in summer. Black

546   bars represent cropped soils, grey bars represent non-cropped soils, and error bars represent

547   standard deviation (n=3).

548   Supplemental Figure 6: The response of soil solution NH_4^+ to increases in KCl extractable NH_4^+,

549   in whole soil microcosms. Soils were incubated at 4ºC for 24 h after the addition of different

550   amounts of NH_3. Panel A represents cropped soils, and panel B non-cropped soils. Symbols

551   designate soils from Pendleton (●), Madras (○), Corvallis (▼), and Klamath Falls (△).

552   Table 1: Soil Physical and chemical properties of soils used in this study
553

Location	Pendleton		Madras		Klamath		Corvallis	
Land use	Non-cropped	Cropped	Non-cropped	Cropped	Non-cropped	Cropped	Non-cropped	Cropped
% sand/silt/clay	14.2/71.8/14		38.5/35.7/25.8		83/4/13		19.9/57.5/22.6	
pH	7.26	6.15	7.68	6.87	7.36	6.42	6.18	6.38
WHC -33 kPa[†]	0.45	0.35	0.38	0.39	0.32	0.22	0.26	0.32
Total C (g kg-1)[#]	20.7	10.6	8.7	8.7	13.4	6.6	25.7	12.9
Total N (g kg-1)[#]	1.8	0.9	0.9	0.8	1.1	0.6	1.7	0.6
NH_4^+ summer[‡‡]	3.61	6.8	8.48	11.6	0.56	8.26	2.09	4.18
NH_4^+ winter[‡‡]	3.18	3.1	1.29	2.92	9.54	0.92	1.93	1.44
CEC(meq/100g)[‡]	21.9	15.1	20.5	22.0	13.6	10.7	16.9	14.2
AOA amoA[§]	352±197	123±73	474±47	283±244	419±228	307±48	3.9±2.7[††]	0.9±0.7[††]
AOB amoA[§]	5.9±2.6	5.6±0.9	0.5±0.2	15.6±15	9.4±8.7	9.8±2.1	1.0±0.5[††]	0.8±0.2[††]
N-mineralization[¶]	1.5±2.4	0.7±0.1	1.3±0.4	0.8±0.2	1.0±0.3	1.5±0.3	1.2±0.56	0.5±0.09

554   †: Water holding capacity
555   ‡: cation exchange capacity
556   §: Gene copies 10^6 from Taylor et al. (2013)
557   ¶: NH_4^+ production rates (mg N kg^{-1} DW soil day^{-1})
558   #: Determined by the Central Analytical lab, Oregon State University.
559   †† Gene copies 10^6 from Taylor et al. (2010)
560   ‡‡: Background KCl extractable NH_4^+ mg N kg $^{-1}$ soil

561   Table 2: Background total net nitrification rates

Season	Site	Background Nitrification‡	
		Cropped	Non-cropped
Winter			
	Pendleton	0.37±0.2	0.17±0.3
	Madras	0.76±0.3	0.08±0.8
	Klamath	0.61±0.04	2.8±1.0
	Corvallis	0.60±0.2	1.4±0.13
Summer			
	Pendleton	0.31±0.3	0.37±0.4
	Madras	0.78±1.4	0.92±0.3
	Klamath	1.7±0.2	0.81±0.04
	Corvallis	0.14±0.2	0.59±0.06

562   Means given ± standard deviation
563   † mg NH_4^+ -N kg^{-1} soil
564   ‡Background net nitrification
565