

1 **Gaseous Nitrogen Losses Following Soil Amendment with Biosolids**

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26 **Abstract**

27 Biosolids (the nutrient rich organic matter derived from wastewater treatment processes)
28 are regarded as a valuable source of major plant nutrients, especially nitrogen (N).
29 However, a significant proportion of the biosolids N can be lost as N gases after biosolids
30 application to soil. This paper quantifies gaseous N losses due to ammonia volatilisation
31 (boric acid absorption method) and denitrification (acetylene inhibition method) under
32 controlled conditions in the laboratory. Biosolids (dewatered cake) were mixed with two
33 contrasting soils from subtropical Australia at a rate designed to meet crop N
34 requirements for irrigated cotton or maize (i.e. equivalent to 180 kg N ha⁻¹). In the first
35 experiment, aerobically (AE) and anaerobically (AN) digested biosolids were mixed into
36 a heavy Vertosol soil and then incubated at 30°C with moisture contents ranging from
37 75% to 150% of field capacity. Ammonia volatilization over 72 days accounted for less
38 than 4% of the applied NH₄-N but 24% (AN) to 29% (AE) of the total applied biosolids N
39 was lost through denitrification in 105 days. In the second experiment AN biosolids with
40 and without added polyacrylamide polymer were mixed with either a heavy Vertosol or a
41 lighter Red Ferrosol and then incubated for 98 days at 30°C. Moisture contents ranged
42 from 75% field capacity to saturation during the experimental period. The N loss from
43 denitrification was higher from the Vertosol (16-29% of the total N applied) than the Red
44 Ferrosol (7-10% of the total N applied), while the addition of polymer to the biosolids
45 increased N loss from denitrification from 7 to 10% and from 16 to 29% of the applied N
46 in the Red Ferrosol and Vertosol, respectively. The major product of the denitrification
47 process was N₂ gas, accounting for >90% of the emitted N gases from both experiments.
48 Our findings show that denitrification could be a major pathway of gaseous N losses
49 under warm and moist conditions typical of those found in subtropical Australia.

50

51 Key words: Biosolids; Mineralisation; Denitrification; Ammonia volatilisation; N₂O;
52 Acetylene

53

54 **1. Introduction**

55 The area of agricultural land to which biosolids (the nutrient rich organic matter derived
56 from wastewater treatment processes) is being applied, and the quantities of biosolids
57 applied to cropping land have increased significantly in the last two decades. This
58 increase has been due to both an increasing recognition of the nutrient value of the
59 biosolids and environmental concerns about 'traditional' disposal methods such as
60 landfill. Land application of biosolids has been suggested as a way of replenishing soil
61 organic matter stores as well as supplying nutrients such as nitrogen (N), phosphorus (P),
62 sulphur (S), potassium (K) and essential micronutrients. In addition to plant growth
63 responses to added nutrients, biosolids are also reported to improve soil structure and
64 water holding capacity and have beneficial effects on microbial biomass and activity
65 (Eriksen et al., 1999; Barbarick and Ippolito 2000; Leiffield et al., 2002; Bergkvist et al.,
66 2003; Barry et al., 2004; Bell et al., 2004; Crecchio et al., 2004; Meyer et al., 2004).
67 When biosolids N is added to soil it will undergo a series of the transformations which
68 can add to the pools of plant available mineral N in the soil but also lead to gaseous losses
69 to the atmosphere. The added organic N in the biosolids can be mineralised to NH₄-N and
70 NO₃-N but this newly formed NH₄-N and any initial NH₄-N in the biosolids can be lost
71 via NH₃ volatilisation. The amount of N loss through NH₃ volatilisation will be affected
72 by many factors including the method of biosolids application and subsequent
73 incorporation, the NH₄ -N concentration in the soil and biosolids and the pH of the
74 biosolids and soil (Quemada et al., 1998; He et al., 2003). Additionally, NO₃-N can be
75 lost through denitrification (formation of N₂ and N₂O), with the key soil factors affecting

76 the extent of denitrification activity being air-filled porosity and the concentrations of
77 $\text{NO}_3\text{-N}$ and labile carbon (C) in the soil (Pu et al., 2001). Quantitative information about
78 these N transformations and gaseous loss pathways is essential to develop effective N
79 management strategies so that land application of biosolids minimises any unintended
80 effects on the environment (Vieira et al., 2005).

81 The results from field trials at four different locations covering different soil types and
82 cropping systems in subtropical southeast Qld indicated that a significant amount of
83 applied biosolids N (ranging from 12-40%) could not be accounted for one year after
84 biosolids application (Barry *et al*, 2006). As three of the four sites did not experience
85 runoff or show evidence of leaching losses, it was hypothesised that the unaccounted for
86 N was lost by either denitrification or NH_3 volatilisation. While there have been reports of
87 gaseous N losses from biosolids due to NH_3 volatilisation (Harmel et al., 1997; He et al.,
88 2003; Robinson and Röper, 2003) little has been done to quantify the N lost through
89 denitrification. The difference between the applied and the recovered N from both the
90 plant and soil has usually been attributed to the leaching of NO_3 and volatilisation of NH_3 ,
91 with any N loss unaccounted for by these two processes attributed to denitrification
92 (Currie et al., 2003; Mendoza et al., 2006).

93 The field trials reported by Barry et al. (2006) provided favourable conditions for
94 denitrification losses in that both biosolids and soils were characterised by low C:N ratios,
95 soil textures were mostly medium-heavy clays and high concentrations of $\text{NO}_3\text{-N}$ were
96 found in the soils as a result of rapid mineralisation of organic N from the biosolids (Bell
97 et al., 2004). In addition, the biosolids used in the field trials contained a flocculant
98 polymer. This is often added during biosolids processing to help in the dewatering
99 process. The added polymer, however, can affect the water holding characteristics of the
100 biosolids. We had observed that biosolids with added polymer were normally more sticky

101 and tended to aggregate into lumps, compared to the polymer free biosolids. The
102 biosolids with added polymer tended to lose water more slowly than the surrounding soil
103 during dry periods and also absorb water more quickly after rain. This additional moisture
104 retention, combined with the high mineral N and C concentrations, would favour
105 denitrification activity in, and near, biosolids lumps.

106 The aim of two experiments reported here was to directly quantify gaseous N losses
107 through direct measurement of denitrification and ammonia volatilisation. The impact of
108 variables like soil type, prevailing moisture and biosolids characteristics such as the
109 production process (anaerobic or aerobic) and the presence of added polymer on the
110 extent of these gaseous losses was assessed under controlled temperature conditions.

111

112 **2. Materials and methods**

113 *2.1 Soil and biosolids*

114 Soils (from the top 10cm of the soil profile) used in the experiments were a heavy Black
115 Vertosol collected from Cecil Plains (27.40S, 151.74E, elevation: 400m), and a strongly
116 structured Red Ferrosol from Kingaroy (28.33S, 152.30E, elevation: 507 m). Both soils
117 came from sites in subtropical Queensland, Australia that had been previously used to
118 conduct a series of field trials investigating the effects of biosolids application on crop
119 productivity and the fate of biosolids-derived major nutrients and trace metals (Barry et
120 al., 2006).

121

122 Biosolids (dewatered cake) used in these experiments were sourced from waste water
123 treatment plants located in south east Queensland. In Experiment 1, two biosolids were
124 used - an anaerobically digested product from an urban catchment area and an aerobically
125 digested product from a semi urban catchment area. In Experiment 2, an anaerobically

126 digested product from an urban catchment area (different to the one in Experiment 1) was
127 used.

128

129 *2.2 Experiment 1*

130 The experiment was conducted as a randomised block design with 4 replications. The
131 experiment included a control (Vertosol soil with no added biosolids), in addition to
132 treatments in which soil was amended with aerobically or anaerobically digested
133 biosolids. The biosolids were mixed with sieved (<10 mm) field moist soil at rates
134 equivalent to 20 dry Mg anaerobic biosolid ha⁻¹ (23g dry biosolids kg⁻¹ dry soil) and 14
135 dry Mg aerobic biosolid ha⁻¹ (17 g dry biosolids kg⁻¹ dry soil), these being equivalent to
136 the Nitrogen Limited Biosolids Application Rate (NLBAR) for each biosolids determined
137 using the methods described by NSW EPA (1997). The target crop N requirement to be
138 met at these NLBARs was 180 kg N ha⁻¹, with the assumption that net mineralisation
139 rates were 25% and 15% for the aerobic and anaerobic biosolids, respectively. Biosolids
140 lumps >25mm diameter were removed prior to mixing.

141

142 The moist soil (control) and the mixtures of moist soil and added biosolids were packed
143 to a height of 100 mm in PVC cylinders with an inner diameter of 125 mm and height of
144 150 mm. The bottoms of the cylinders were sealed using a PVC saucer with a 5mm hole
145 in the centre that was sealed by a silicon septum. A PVC cover with an O-ring fixed on
146 the inside was screwed to the top of the cylinder. A hole of 5 mm diameter was drilled in
147 the centre of the top cover, with the hole able to be sealed with a silicon septum.

148 Additional sets of the cylinders were prepared in the same way for each treatment to
149 allow the temporal variation in background NO₃-N in the soil to be monitored, although
150 only two replications were established for this purpose.

151

152 The packed soil cores were wetted to 75% of field capacity, determined on the basis of
153 gravimetric moisture content measured on the same soils in the field, and then kept in an
154 incubator at a constant temperature of $30\pm 1^{\circ}\text{C}$. On days 14, 28 and 42 after
155 commencement, moisture content was increased to field capacity by adding the required
156 amount of deionised water and on day 77 moisture content was further increased to 150%
157 of field capacity. After each wetting, soil was allowed to gradually dry down, although
158 from the days 1 to 42 and 77 to 85, the covers were left on with the septums removed to
159 reduce the rate of moisture loss. For the remainder of the experiment, cylinders were left
160 open except during periods of gas sampling.

161

162 Soil cores in the additional sets of cylinders were sampled 14, 21, 28, 42, 56 and 70 days
163 after the incubation started by removing two cylinders per treatment at each time, and soil
164 $\text{NO}_3\text{-N}$ was determined on the moist soil. After 105 days, all remaining soil cores in the
165 cylinders were taken out and re-mixed. Half of the soil from each cylinder was kept moist
166 prior to analysis for mineral N. The remaining soil was oven dried at 40°C and ground to
167 pass a 2 mm sieve before conducting other chemical analyses.

168

169 *2.3 Experiment 2*

170 Experiment 2 was also conducted using a randomised block design but with only 3
171 replications. The treatments consisted of the unamended Black Vertosol or Red Ferrosol
172 soils (controls), as well as the soils amended with an anaerobically digested biosolids
173 with and without an added polymer (cationic flocculant type FO 4490 supplied by SNF
174 Australia Pty Ltd). The biosolid application rate was determined by the same method as

175 described in experiment 1, and also designed to mineralise the equivalent of 180 kg
176 available N ha⁻¹.
177
178 Procedures and materials in Experiment 2 were similar to those used in Experiment 1
179 except for the following details. The cylinders used in Experiment 2 were slightly
180 smaller, with an inner diameter of 85 mm. The moisture regimes imposed on the soil
181 cores inside the cylinders were adjusted to 75% of Field Capacity on days 1 and 12, 100%
182 Field Capacity on days 21 and 51, and 150% of Field Capacity on day 52. After each
183 wetting adjustment, soil was allowed to gradually dry down. Moisture contents were
184 achieved by weighing the cores and adding deionised water to achieve the target
185 gravimetric moisture content. Except for days 21 to 52 when the cylinder covers were left
186 on with the septums removed, cylinders were left open with PVC covers removed to
187 facilitate gas exchange after gas samples were actually being collected. Soil NO₃-N status
188 was again monitored using soil cores in the additional sets of cylinders, with sampling
189 undertaken 14, 28 and 42 days after the incubation period started. The experiment was
190 terminated once gaseous N emissions dropped to insignificant levels.

191

192 *2.4 Ammonia volatilisation determination*

193 A PVC container with 5 ml of 4% boric acid was attached to the inside wall of each
194 cylinder containing the various soil and soil-biosolid mixtures, after which cylinders were
195 sealed and returned to the incubator for periods ranging from 4 to 48 hours. The NH₃-N
196 entrapped in the boric acid was determined by titration with 0.0025 M H₂SO₄, with the
197 amount of NH₃-N absorbed in the boric acid for any measurement period calculated using
198 the following relationship –

$$199 \quad \text{NH}_3\text{-N} = (S-C) \times T,$$

200 where S was the volume of H₂SO₄ used to titrate the sample in ml;
201 C was the volume used to titrate the blank control in ml; and
202 T was the titre of the titrant, with T for 0.0025 M H₂SO₄ taken as 70 µg N ml⁻¹ (Khan et
203 al., 1997).

204 The measurements of ammonia volatilisation were terminated on day 72, when the
205 emissions dropped below detection limits. The accumulated amount of volatilised NH₃-N
206 over the experimental period was calculated using the trapezoidal integration method.

207

208 *2.5 N₂O gas determination*

209 Denitrification losses were assessed in all soil and soil-biosolids treatments with and
210 without added acetylene gas during the incubation period. Acetylene was added to inhibit
211 the transformation of N₂O into N₂ and ensure all N losses from denitrification were
212 captured. After sealing the cylinders with the covers, the 'plus acetylene' treatments had
213 the gas injected into the cylinders at 8% (v/v) of the air phase. All cylinders were then
214 kept in the incubator at 30°C and gas samples were collected after periods of 12 to 48
215 hours using a 12ml pre-evacuated glass tube. The concentration of N₂O in the gas
216 samples was determined using a Varian CP-3800 GC (Varian, Netherlands). The
217 accumulated N₂O-N lost over the measurement period was calculated in a similar fashion
218 to the cumulative NH₃ volatilisation losses, using the trapezoidal integration method.

219

220 *2.6 Soil and biosolids chemical analyses*

221 Standard soil analyses were conducted using methodology outlined in Rayment and
222 Higginson (1992). Briefly, pH and EC of both soil and biosolids were determined in
223 water using a 1:5 soil/biosolids to water ratio. Total K, P and S concentrations in soil and
224 biosolids were determined by inductively coupled plasma atomic emission spectroscopy

225 (ICPAES) following digestion using a concentrated acid mixture of 3:1 HNO₃:HCl
226 (volume).
227 Total soil and biosolids C concentrations were determined by a dry-combustion method
228 using a LECO CNS-2000 analyser (LECO Corporation, MI, USA). The total N
229 concentration of both soils and biosolids was determined by the semi-micro Kjeldahl
230 digestion method (Bremner and Mulvaney 1982). Biosolids and soil mineral N (NH₄ and
231 NO₃) was extracted using a 1:10 ratio of biosolids/soil:2M KCl solution, and then
232 determined by an automated colorimetric method (Method 7C2, Rayment and Higginson,
233 1992). The quantity of NO₃-N determined by this method included both nitrite N and
234 nitrate N.

235

236 *2.7 Denitrification capacity and potential*

237 Soil denitrification capacity (DC) can be defined as the ability of a soil to reduce NO₃-N
238 due to the action of denitrifying bacteria already in the soil under anaerobic conditions.
239 Soil denitrification capacity can reach its maximum, defined as the soil denitrification
240 potential (Yeomans et al., 1992), when available organic carbon is unlimited.

241

242 The denitrification capacity for soils and soils/biosolids mixtures was estimated by adding
243 14 ml of water and 1 ml of KNO₃ solution (1.38 mg N ml⁻¹) to 4.5 g oven dried soil (<2
244 mm) sealed in a 60 ml syringe. The denitrification potential for the same treatments was
245 determined by adding 13 ml of water, 1 ml of KNO₃ solution (1.38 mg N ml⁻¹) and 1 ml
246 sucrose solution (1.36 mg C ml⁻¹) to the same amount of oven dried soil in the 60 ml
247 syringes. After expelling all the air, syringes were sealed and incubated at 40°C for 7
248 days. Soil nitrate before and after incubation was then analysed and the denitrification
249 capacity was calculated as

250 $DC = 100 \times ([NO_3-N]_{initial} - [NO_3-N]_{final} / [NO_3-N]_{initial}).$

251

252 *2.8 Statistical analysis*

253 The differences between treatment means were tested for statistical significance using the
254 least significant difference (LSD) procedure in the Genstat® statistical package (8th
255 Edition). The figures were produced using Microsoft® Excel 2000 with standard error
256 bars presented in the relevant figures.

257

258 **3. Results**

259 *3.1 Characterisation of the soils and biosolids*

260 Selected properties of the Black Vertosol and Red Ferrosol soils (Table 1) show that
261 while both soils contained similar proportions of silt + clay (viz. 86% and 79% for the
262 Vertosol and Ferrosol, respectively), the gravimetric soil moisture content at field
263 capacity was much higher for the Vertosol (50%) than the Ferrosol (29%). In addition, the
264 Vertosol contained lower concentrations of both C and TKN, and had a higher pH, EC
265 and C:N ratio than the Ferrosol (Table 1). Little NO₃-N and NH₄-N were found in either
266 of the soils before the incubation.

267

268 The TKN of the biosolids ranged from 5.9% (anaerobic biosolid B without polymer) to
269 6.5% (anaerobic biosolid A with polymer; Table 2). Concentrations of NO₃-N were low
270 in all biosolids used, but concentrations of NH₄-N were much higher, ranging from 4%
271 (anaerobic biosolid B without polymer) to 19% (anaerobic biosolid A with added
272 polymer) of the total biosolids N. In Experiment 2, the concentrations of both NH₄-N and
273 TKN were lower in the polymer free anaerobic biosolids (2500 mg kg⁻¹ and 5.9%,
274 respectively) than in the same biosolid with added polymer (5300 mg kg⁻¹ and 6.2%,

275 respectively), probably due to gaseous N losses during the extended period on drying
276 beds required for the polymer free biosolids. The higher $\text{NH}_4\text{-N}$ concentration found in
277 the anaerobic biosolids ($12,200 \text{ mg kg}^{-1}$) compared to the aerobic biosolids ($5,600 \text{ mg kg}^{-1}$)
278 ¹⁾ was consistent with findings of a US survey of over 150 waste water treatment plants
279 reported by Sommers (1977). The much higher mean $\text{NH}_4\text{-N}$ concentrations in
280 anaerobically digested biosolids compared to those produced in aerobic processes were
281 attributed to much reduced NH_3 volatilisation losses during the anaerobic digestion
282 process. All biosolids contained significant amounts of C, which ranged from 33-37% on
283 a dry weight basis, and very low C:N ratios (5 – 6, Table 2).

284

285 *3.2 Temporal changes in $\text{NO}_3\text{-N}$ concentrations*

286 During experiment 1, $\text{NO}_3\text{-N}$ concentrations in the Vertosol reached an early peak 14
287 days after commencement of the incubation (170 and $150 \text{ mg NO}_3\text{-N kg}^{-1}$ soil for the
288 aerobic and the anaerobic biosolids-amended soils, respectively) and then rapidly
289 declined to reach a minimum on day 28 (70 and $55 \text{ mg NO}_3\text{-N kg}^{-1}$ soil for the same
290 treatments respectively; Fig. 1a). After this minimum was reached, $\text{NO}_3\text{-N}$ concentrations
291 then increased continuously until day 70 for the soil amended with aerobic biosolids and
292 until the end of the experiment on day 105 for the soil amended with anaerobic biosolids.
293 At the end of the 105 day incubation period, the $\text{NO}_3\text{-N}$ concentrations were 150 and 170
294 $\text{mg NO}_3\text{-N kg}^{-1}$ soil for the aerobic and anaerobic biosolids-amended soils respectively.
295 The same general pattern of fluctuations in $\text{NO}_3\text{-N}$ was found in the control treatment
296 (soil without added biosolids), although concentrations were much lower, differing by \geq
297 100 mg kg^{-1} at both the very early and late stages of the incubation period.

298

299

[insert Fig. 1a, 1b here]

300

301 There were similar fluctuations in $\text{NO}_3\text{-N}$ concentrations in the Vertosol soil amended
302 with the anaerobic biosolids in Experiment 2, where concentration peaked on day 28,
303 decreased sharply until day 40 and then increased until the end of the incubation period
304 (Fig.1b). The pattern in the Vertosol control treatment (no added biosolid) differed
305 somewhat, with a slow early increase in $\text{NO}_3\text{-N}$ concentration that accelerated during the
306 middle of the incubation period but declined slightly from day 40 to day 98. Although not
307 significant, the $\text{NO}_3\text{-N}$ concentration in the Vertosol mixed with polymer-added biosolid
308 ($85\text{-}130 \text{ mg NO}_3\text{-N kg}^{-1}$) was consistently lower than in the same soil mixed with
309 polymer-free biosolids ($98\text{-}147 \text{ mg NO}_3\text{-N kg}^{-1}$) from day 28 until the end of the study.

310

311 In the Red Ferrosol soil this trend for consistently lower $\text{NO}_3\text{-N}$ concentrations in soil
312 mixed with polymer-added biosolids ($150\text{-}180 \text{ mg NO}_3\text{-N kg}^{-1}$ soil) compared to that in
313 soil mixed with polymer-free biosolids ($180\text{-}210 \text{ mg NO}_3\text{-N kg}^{-1}$ soil) was also observed,
314 particularly after day 40 (Fig. 1b). However trends differed between the two soil types in
315 earlier periods of incubation where there was no peak concentration at day 28 for either
316 polymer treatment (as found in the Vertosol soil) but a consistent increase in $\text{NO}_3\text{-N}$
317 concentration over the whole incubation period. At the end of the incubation period,
318 significantly higher $\text{NO}_3\text{-N}$ concentrations were found in the biosolid-amended Ferrosol
319 soil ($184\text{-}210 \text{ mg NO}_3\text{-N kg}^{-1}$) than in the biosolid-amended Vertosol ($130\text{-}147 \text{ mg NO}_3\text{-}$
320 N kg^{-1}), irrespective of polymer treatment.

321

322 *3.3 Ammonia volatilisation*

323 Ammonia volatilisation was low during the 72 day monitoring period in Experiment 1,
324 accounting for <4% of the applied $\text{NH}_4\text{-N}$ (Fig. 2), with most of the NH_3 volatilised

325 during the first few days. Subsequent wetting and drying cycles had little effect on
326 cumulative NH₃ volatilisation. Soil amended with anaerobically digested biosolids lost
327 more N through NH₃ volatilisation, in absolute terms, than soil amended with biosolids
328 produced under aerobic conditions. This was not surprising given the higher initial
329 concentrations in the anaerobic biosolids (Table 2). The addition of acetylene gas led to a
330 slight but significant increase in NH₃ volatilisation. Volatilisation losses of NH₃ were not
331 measured in Experiment 2, as the results from Experiment 1 indicated total N loss by this
332 pathway was small.

333

334 [insert Fig. 2 here]

335

336 *3.4 Denitrification*

337 Significant amounts of the applied biosolids N were lost through denitrification during
338 Experiment 1 (Fig. 3), with the percentage N loss higher from the soil amended with the
339 aerobically digested biosolids (~30%) than from the soil amended with the anaerobically
340 digested biosolids (24%). This was despite the fact that the absolute amount of N lost was
341 higher from the soil mixed with anaerobically digested biosolids (0.26g N cylinder⁻¹) than
342 from that mixed with aerobically digested biosolids (0.20g N cylinder⁻¹), which reflected
343 the total amounts of biosolids N added.

344

345 [insert Fig. 3 here]

346

347 There were big differences in the N₂O gas emissions between treatments in which
348 acetylene gas was either added (24-29% total added N denitrified) or omitted (~2% total
349 added N denitrified) during the measurement periods (Fig. 3) in Experiment 1. Based on

350 the difference between the N₂O gas emitted from treatments with and without acetylene it
351 could be concluded that the predominant form of denitrified N was as N₂ (>90%) rather
352 than N₂O (<10%), and that there was no difference in the ratios of N₂O/N₂ generated from
353 the Vertosol soil amended with the two types of biosolids.

354

355 Cumulative denitrification losses during Experiment 1 accrued by way of a series of
356 stepwise increases that corresponded to the various wetting events during the incubation,
357 indicating the key role played by soil water content on denitrification activity (Fig. 3).

358 The first significant denitrification activity started 14 days after the incubation period
359 started when the soil water content was raised to 75% of field capacity. Significant
360 increases in denitrification losses were subsequently observed when the soil/biosolids
361 mixtures were rewetted on days 42 (100% field capacity) and 77 (150% field capacity).

362

363 The patterns of denitrification activity were similar for both the heavy Vertosol and the
364 lighter Red Ferrosol (Fig. 4 a and b respectively) in Experiment 2. Losses were low in the
365 first 3 weeks and then started increasing from day 24, following the raising of soil
366 moisture content to field capacity. However by far the greatest increase in denitrification
367 activity occurred on day 52, just a few days after the moisture content of the cores was
368 raised to 150% field capacity. Subsequent activity was minimal as the soil cores were
369 allowed to dry.

370

371 [insert Fig 4 here]

372

373 Interestingly, the proportional N losses from denitrification recorded in the Vertosol soil
374 amended with polymer-added biosolids in both Experiments 1 and 2 were similar,

375 ranging from 24-30% of the applied N irrespective of biosolids type. The cumulative N
376 loss was much lower (16% of applied biosolids N) for the Vertosol soil in which the
377 anaerobic biosolids without polymer were added (Fig 4a). The N lost through
378 denitrification was much lower in the lighter Red Ferrosol compared to the Vertosol for
379 both the polymer-added and polymer-free treatments (Fig. 4b). While the presence of
380 polymer in biosolids added to the Red Ferrosol had a much smaller impact on
381 denitrification losses than in the Vertosol soil, the loss of 10% of applied biosolids N
382 denitrified in the presence of polymer compared to 7% biosolids N denitrified in the
383 absence of polymer was still significant. Consistent with the findings from Experiment 1,
384 comparisons of losses with and without added acetylene suggested that the dominant
385 denitrification product in both soil types was N₂ gas, representing 85 to 95% of the total
386 N lost by this process.

387

388 *3.5 N loss determined by mass balance*

389 In Experiment 1, 55-66% of the applied biosolids N could be recovered in the soil at the
390 end of the incubation period, leaving 34-45% of the applied biosolids N unaccounted for
391 (Table 3). The gas measurements suggest that most of this loss was probably via
392 denitrification activity, as NH₃ volatilisation accounted for <4% of the applied NH₄-N, or
393 <1% of the total applied biosolids N. In the treatments receiving aerobic biosolids
394 applications, the N loss measured by the mass balance method (34% of applied N) was
395 similar to the combined gaseous emissions (29% of applied N, Fig.3), but for the
396 treatment receiving anaerobic biosolids the N loss measured by the gas emissions (24% of
397 applied N) was much lower than that measured by mass balance (43% of applied N,
398 Fig.3).

399

400

[insert Table 3 here]

401

402 Mass balances of applied N from Experiment 2 indicated that 29-36% (Black Vertosol)
403 and 15-20% (Red Ferrosol) of the applied biosolids N could not be accounted for from
404 cylinders with added acetylene at the end of the incubation period. Although NH₃
405 volatilisation measurements were not carried out in this experiment, the relative
406 unimportance of this loss pathway in Experiment 1 suggests the apparent N loss was most
407 likely due to denitrification. The apparent N loss was similar in cylinders with (15-36%
408 of applied N) or without (14-40% of applied N) added acetylene for both soil types, but
409 the presence of polymer in the biosolids increased denitrification losses from 14-33% of
410 applied N to 20-40% of applied N for both soil types. The apparent N loss from cylinders
411 with added acetylene determined by the mass balance method (15-36%) was notably
412 higher than that measured by the gas emissions (7-29%), which was consistent with
413 observations in Experiment 1.

414

415 *3.6 Denitrification capacity and potential*

416 The Vertosol soil lost 58% of the applied NO₃-N after 7 days of incubation at 40°C in the
417 absence of the sucrose C source, but losses reached 100% when the sucrose-C was added
418 (Table 4). Losses were lower in the Red Ferrosol, with only 20% of the applied NO₃-N
419 lost without the added sucrose-C and only 66% lost when the sucrose-C was added.

420

421

[Insert Table 4 here]

422

423 **4. Discussion**

424 While biosolids contain significant amounts of potentially available nutrients like N, these
425 experiments also provide clear evidence that appropriate management strategies will be
426 needed to ensure biosolids N is retained in soil for crop/pasture use, rather than lost to the
427 atmosphere. The apparent N losses due to NH₃ volatilisation represented only a very
428 small proportion of both the total applied N and the biosolids N that could not be
429 accounted for at the end of the incubation period (Fig. 2). While the quantities of N
430 volatilised were greater in the anaerobically digested biosolids, this was not unexpected
431 due to the greater rate of total N addition (Table 3) and that approx. 20% of the total N
432 added was already in the form of NH₄-N (Table 2).

433
434 In an incubation study for 62 days at 21°C, Quemada et al. (1998) reported that soil
435 receiving surface applied biosolids lost 71% of the applied NH₄-N, but when the same
436 biosolid was incorporated into the soil losses fell to < 5%. Similarly, He et al. (2003)
437 found that after an incubation period of 180 days the N loss due to NH₃ volatilisation
438 accounted for 18% of the applied mineral N when biosolids were applied to the soil
439 surface but less than 4% when biosolids were incorporated. Significant NH₃ volatilisation
440 has also been reported from field studies in which biosolids were applied to the field
441 without incorporation, with Robinson and Röper (2003) showing that 44-55% of the
442 applied biosolids NH₄-N was lost within 14 days of biosolids application. These large
443 losses occurred despite the experiment being conducted under weather conditions
444 unfavourable for volatilisation (low temperature, high humidity and little wind).

445
446 The low NH₃ volatilisation losses observed in Experiment 1, in which biosolids were
447 incorporated into soil, were consistent with other incubation studies which showed much
448 reduced losses after biosolids were incorporated into soil rather than surface applied. This

449 finding provides a strong incentive to retain the current licensing requirement to
450 incorporate land-applied biosolids within 36 hours of application (NSWEPA, 1997). If
451 these requirements are adhered to, NH₃ volatilisation might not be an important pathway
452 for gaseous N losses from biosolids-amended soils.

453

454 In contrast to volatilisation, biosolids N loss due to denitrification could represent
455 significant proportions of the total N applied. Treatment impacts on denitrification losses
456 in these two experiments indicated that denitrification rates were regulated by two key
457 factors. These were the concentration of NO₃-N (controlled by mineralisation activity)
458 and the air-filled porosity. Soil water status can be used as a surrogate for the latter factor.
459 While the addition of sucrose was still able to accelerate denitrification activity (Table 4),
460 the availability of a C substrate to sustain microbial activity was unlikely to be a
461 limitation as biosolids contained approx. 35% organic C with low C:N ratios (5-6; Table
462 2).

463

464 In Experiment 1 (Fig. 3), the very low level of gaseous N emissions during the early stage
465 of the incubation could be attributed to the low initial NO₃-N concentration, as little
466 nitrate was found in either the soil or the biosolids before incubation (Tables 1 and 2).
467 Mineralisation activity seemed to have started quickly once the biosolids were applied
468 under the warm and moist conditions of the incubation, as the NO₃-N concentration
469 quickly rose to > 100 mg kg⁻¹ 14 days after commencement for both anaerobic and
470 aerobic biosolids (Fig. 1a). The first big flush of denitrification came with the combined
471 effects of accumulated NO₃-N and elevated soil moisture content (100% field capacity on
472 day 14) in the biosolids treated soils. It is likely that the denitrification activity may have
473 occurred mainly in or near the applied biosolids lumps. Here the required anaerobic

474 conditions for the denitrifying bacteria could be satisfied, the highest concentrations of
475 mineralised $\text{NO}_3\text{-N}$ were likely to be located and there would have been plentiful supplies
476 of labile C. The decreases in soil $\text{NO}_3\text{-N}$ concentrations from day 14 to 28 (Fig. 1) were
477 consistent with increased denitrification losses during the same period (Fig. 3). Although
478 concentrations of $\text{NO}_3\text{-N}$ increased between days 28 and 72, there were rapid increases in
479 denitrification losses with increased soil moisture on day 42 (100% field capacity) and
480 day 80 (150% field capacity). These flushes of denitrification therefore corresponded to
481 periods of low air-filled porosity, but could not be discerned in the net nitrification trend
482 evident over the same period.

483

484 Quantitative information about the N lost via denitrification following land application of
485 biosolids has been rare, and if gaseous N losses were observed most of the N loss was
486 attributed to volatilisation of NH_3 and leaching (Currie et al., 2003; Robinson and Röper,
487 2003). However, in those studies the leached and volatilised N losses often could not
488 account for the quantum of biosolids N lost, and it was hypothesised that these additional
489 N losses were probably due to denitrification (Currie et al., 2003; Mendoza et al., 2006).

490 The findings from our incubation experiments support denitrification as an important
491 pathway for N losses when biosolids are applied to land under warm and moist
492 conditions. The rate of mineralisation of biosolids N, and in particular the rate of
493 nitrification, are likely to play a key role in regulating potential denitrification losses in
494 biosolids-amended soils.

495

496 In both experiments the impact of acetylene addition for increasing the detection of
497 denitrification losses was illustrated, with denitrification almost undetectable unless
498 acetylene gas was added to inhibit the conversion of N_2O to N_2 . This large impact of

499 acetylene allowed us to conclude that N_2 gas was the major product of the denitrification
500 activity, as the N_2O generated in the absence of acetylene accounted for less than 10% of
501 the total emitted N gases (N_2 and N_2O). Weier et al. (1993) reported that high soil water
502 content (i.e. low air-filled porosity) and high NO_3-N concentrations in soil favoured N_2
503 generation from the denitrification process, while Mathieu et al. (2006) found that large
504 stores of labile organic C in soil were also conducive to N_2 generation. Pu et al. (1999)
505 found that N_2 was the dominant product of the denitrification activity (99%) after
506 applying enriched $NO_3-^{15}N$ to a similar Black Vertosol soil in Queensland under the
507 waterlogged conditions.

508
509 The estimations of N loss by the mass balance method (Table 3) suggested that while the
510 addition of acetylene gas had greatly increased detection of denitrification losses, this
511 method was still not able to detect all gaseous N losses (presumably additional
512 denitrification) that occurred in these studies. The results from Experiment 1 showed total
513 N losses averaged 44% of the applied N for the treatments receiving anaerobically
514 digested biosolids, and 36% of the applied N for the treatments receiving aerobically
515 digested biosolids. These losses compared to total measured gaseous losses (volatilisation
516 of NH_3 plus denitrification of N_2O) equivalent to 25% (anaerobic) and 31% (aerobic) of
517 applied biosolids N, respectively. The smaller N loss estimates by the acetylene inhibition
518 method have previously been attributed to incomplete inhibition of the conversion from
519 N_2O to N_2 (Watts and Seitzinger, 2000) and the entrapment of N_2O in the soil profile
520 (Weier et al., 1993; Mahmood et al., 1999).

521

522 The effects of polymer addition during the biosolids dewatering process on the gaseous N
523 emissions were apparent in both the heavy Vertosol and the strongly structured Red

524 Ferrosol, with more applied biosolids N lost from soils treated with the biosolids-polymer
525 combination (Fig.4). This response could be attributed largely to the increased moisture
526 holding capacity, moisture retention and lumpiness of the biosolids-polymer mix. If the
527 amount of biosolids N lost (Fig 4 and Table 3), combined with the net residual mineral N
528 in the soil, are a reflection of the N mineralised from organic sources in the biosolids, it
529 may also be possible that the net mineralisation rate was higher in soils amended with the
530 biosolids-polymer mix – perhaps by maintaining adequate moisture around the biosolids
531 lumps for longer in the dry down process. This would cause more rapid increases in NO₃-
532 N concentrations in the soils amended with the polymer-biosolids mix, hence increasing
533 the risk of denitrification. This issue requires further investigation.

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624 **Table 1.** Selected soil properties (0-10cm depth) used in the experiments

Soil	pH	EC	NH ₄ -N	NO ₃ -N	K _{total}	P _{total}	S _{total}	C _{total}	TKN	Sand	Silt	Clay	C:N
	1:5 H ₂ O	mS cm ⁻¹	mg kg ⁻¹		%							ratio	
Red Ferrosol	6.2	0.05	19	19	0.2	0.08	0.03	1.85	0.2	22	19	60	9.3
Black Vertosol	8.1	0.12	5	15	0.43	0.03	0.01	1.29	0.08	14	15	71	16.1

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637 **Table 2.** Selected chemical properties of biosolids used in the experiments

Biosolid description	pH	EC	Total solids	C _{total}	TKN	P _{total}	K _{total}	S _{total}	NH ₄ -N	NO ₃ -N	C:N ratio
	1:5 H ₂ O	mS cm ⁻¹			%				mg/kg ⁻¹ dry matter		
Experiment 1											
Aerobic (polymer added)	6.5	199	12	33.5	6	4.3	1	0.8	5600	10	5.6
Anaerobic A (polymer added)	7.6	681	18.7	33	6.5	3.5	0.3	1.4	12200	20	5.1
Experiment 2											
Anaerobic B (polymer added)	8.1	431	12.2	36.7	6.2	1.5	0.2	1.5	5300	15	6
Anaerobic B (no added polymer)	8.1	431	11.8	36.7	5.9	1.5	0.2	1.5	2500	15	6.2

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638 **Table 3.** Fate of applied N 105 days (Experiment 1) or 98 days (Experiment 2) after
 639 amending soils with biosolids and incubating at 30°C. Data represent the means of 4 (Experiment 1)
 640 or 3 replicates (Experiment 2), respectively.

	Applied N		Net recovered N				Lost N	
	N _{total}	N _{mineral}	N _{total}	N _{mineral}	N _{organic}	N _{total}		
	mg N		mg N			% applied N	% applied N	
Experiment 1								
Anaerobic Acetylene+	1057	200	603	89	514	57.1	42.9	
Aerobic Acetylene+	684	64	450	55	395	65.8	34.2	
Anaerobic Acetylene-	1057	200	580	59	521	54.9	45.1	
Aerobic Acetylene-	684	64	409	41	368	59.8	40.2	
Lsd (P<0.05)	ND	ND	111	35	114	12.1	12.1	
Experiment 2								
Black Vertosol								
Polymer+ Acetylene+	485	42	311	58	253	64.0	36.0	
Polymer - Acetylene+	473	21	337	39	298	71.3	28.7	
Polymer+ Acetylene-	485	42	290	31	260	59.8	40.2	
Polymer- Acetylene-	473	21	314	36	278	66.4	33.6	
Red Ferrosol								
Polymer+ Acetylene+	485	42	388	187	201	80.0	20.0	
Polymer - Acetylene+	473	21	400	102	298	84.6	15.4	
Polymer+ Acetylene-	485	42	369	171	198	76.0	24.0	
Polymer- Acetylene-	473	21	405	130	275	85.7	14.3	
Lsd (P<0.05)	ND	ND	64	24	65	13.4	13.4	

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642 **Table 4** Denitrification capacity (no C added) and potential denitrification capacity (sucrose-
 643 C added at 0.30 g C kg⁻¹ soil) of a Black Vertosol and a Red Ferrosol incubated under
 644 anaerobic conditions for 7 days at 40°C

Soil	Initial NO ₃ -N		Recovered NO ₃ -N		Denitrification losses	
	mg N kg ⁻¹ soil					
					% applied N	
	Soil	Added	No added C	C added	No added C	C added
Black Vertosol	2	305	130	1	58	400
Red Ferrosol	3	305	245	105	20	66

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662 **Figure captions**

663 Fig. 1 Temporal changes in soil $\text{NO}_3\text{-N}$ concentration during incubation at 30°C for (a) Black
664 Vertosol soil amended with anaerobic or aerobic biosolids in experiment 1 and (b) Black
665 Vertosol and Red Ferrosol soils amended with anaerobic biosolids in experiment 2.

666 Values are the means of two replications, except for data collected at the end of the incubation period when
667 there are four and three replications for experiments 1 and 2, respectively.

668 Different soil types are designated as CP (heavy black Vertosol) and Kry (Red Ferrosol).

669

670 Fig. 2 Cumulative N loss via ammonia volatilisation during a 72-day incubation at 30°C ,
671 from a Black Vertosol soil amended with anaerobic or aerobic biosolids in the presence or
672 absence of acetylene gas during the measurement period.

673 AN+ and AN- indicate anaerobic biosolids with and without acetylene, while AE+ and AE- indicate similar
674 treatments with aerobic biosolids.

675

676 Fig. 3 Cumulative N_2O emissions during a 105-day incubation period at 30°C from a Black
677 Vertosol soil amended with anaerobic and aerobic biosolids in the presence or absence of
678 acetylene gas during the measurement period.

679 AN+ and AN- indicate anaerobic biosolids with and without acetylene, while AE+ and AE- indicate similar
680 treatments with aerobic biosolids.

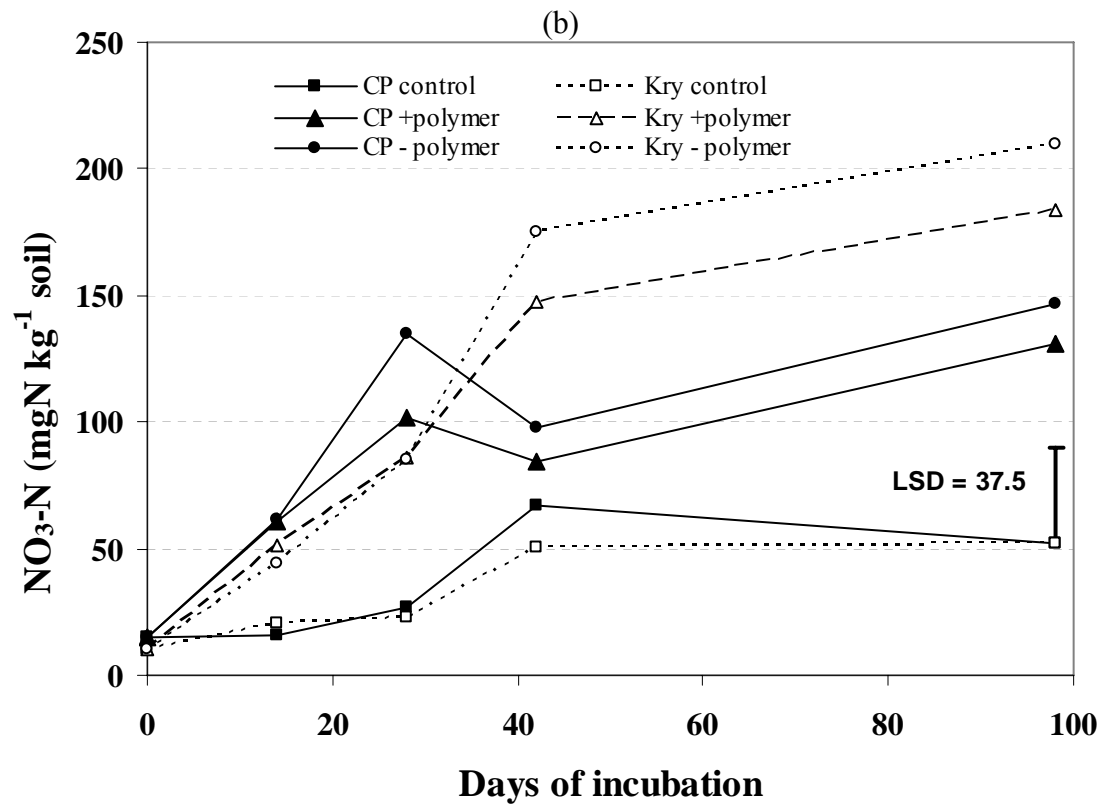
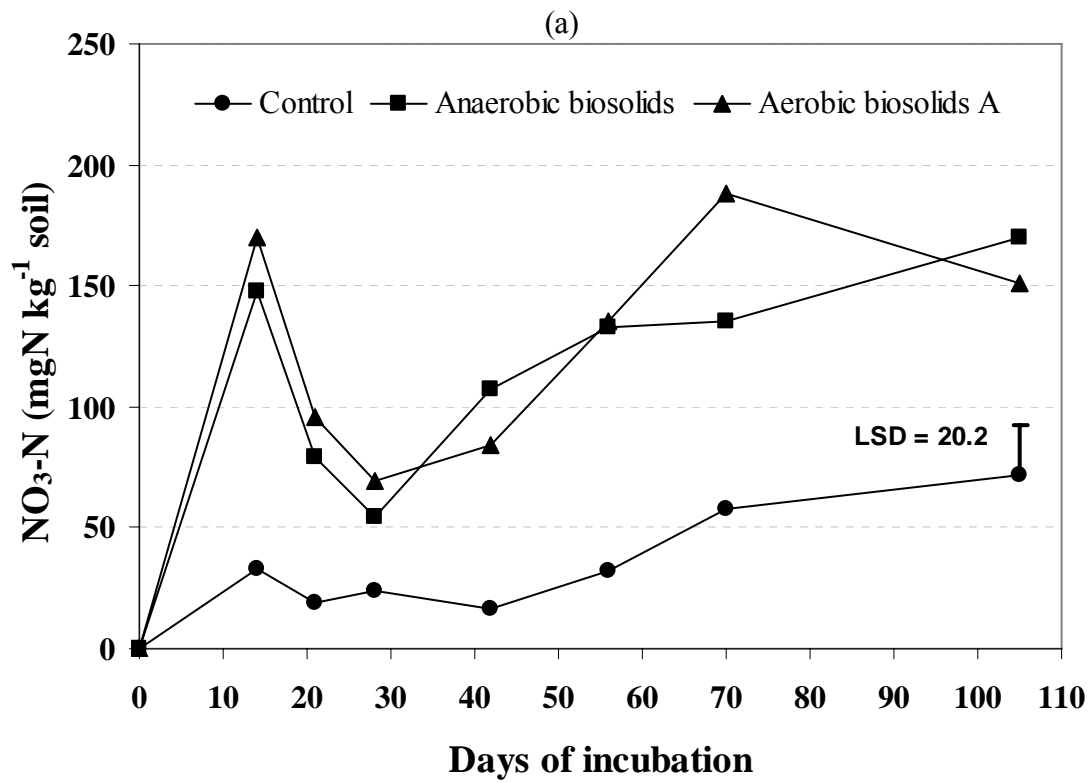
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682 Fig. 4 Cumulative N_2O emissions during a 98-day incubation period at 30°C from (a) Black
683 Vertosol soil amended with anaerobic biosolids with and without added polymer and in the
684 presence or absence of acetylene gas and (b) Red Ferrosol soil amended with anaerobic
685 biosolids with and without added polymer and in the presence or absence of acetylene gas.

686 Poly+ or Poly- indicate presence or absence of polymer in the aerobically digested biosolid.

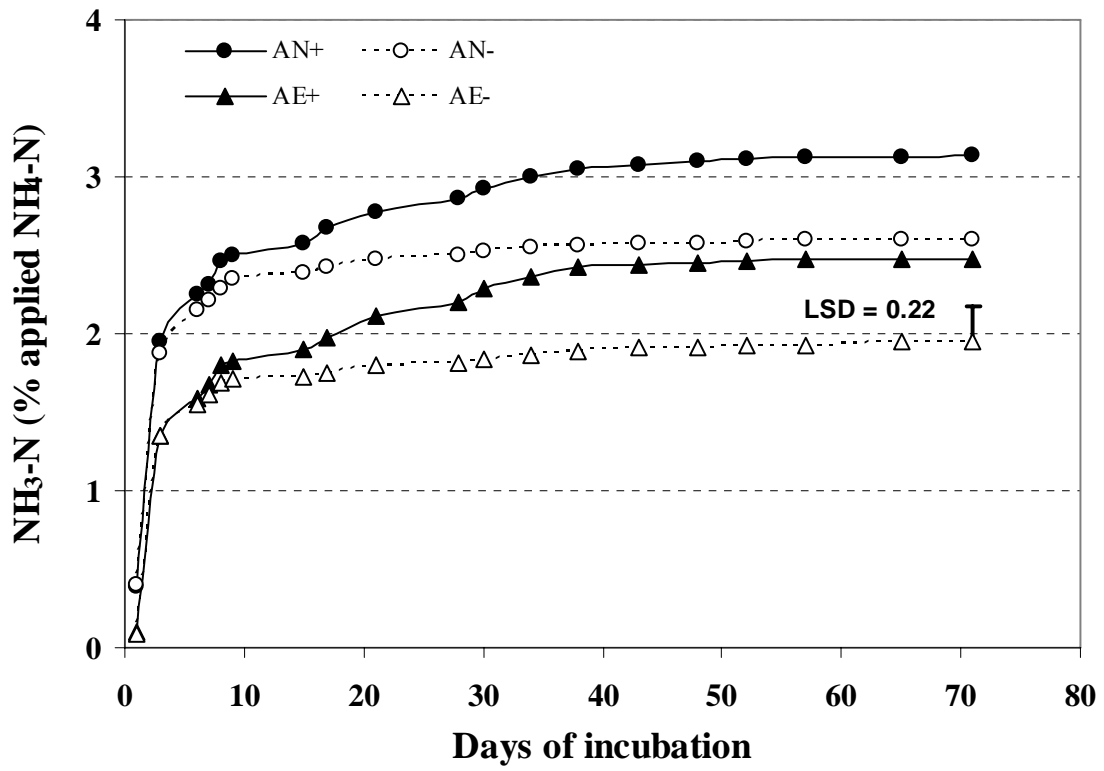
687 Acet+ or Acet- indicate presence or absence of acetylene during the measurement period.

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689 Pu et al., Fig. 1

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691 Pu et al., Fig. 2

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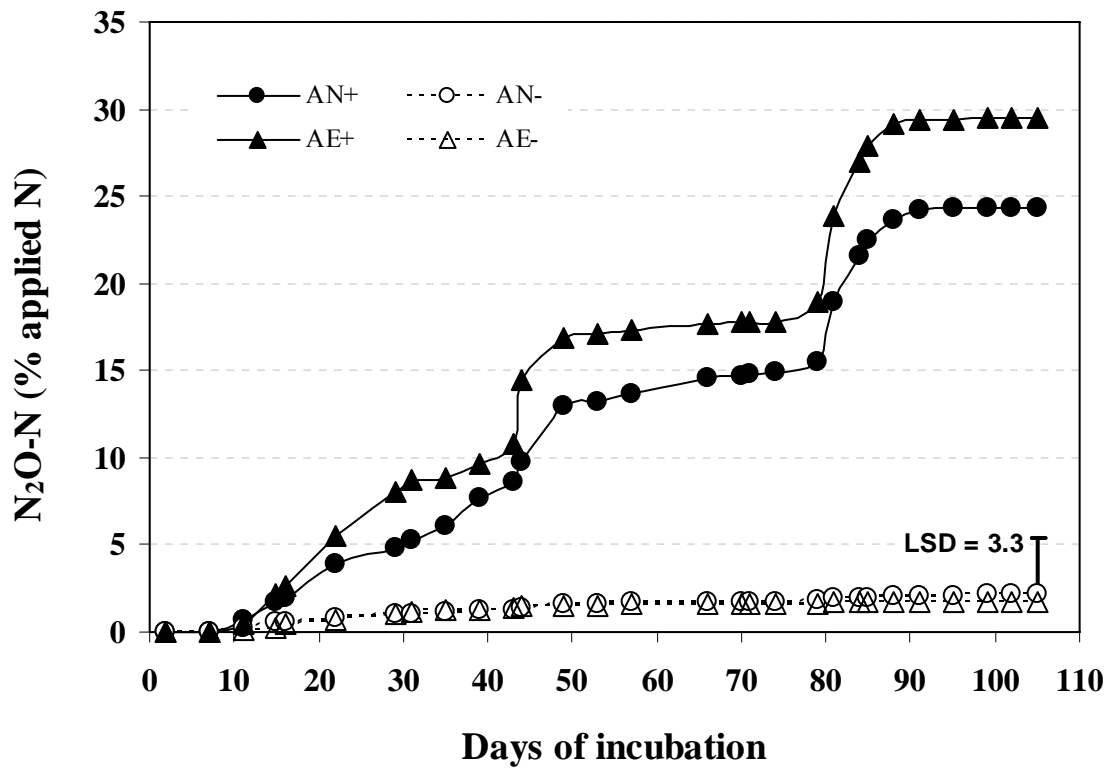
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704 Pu et al., Fig. 3

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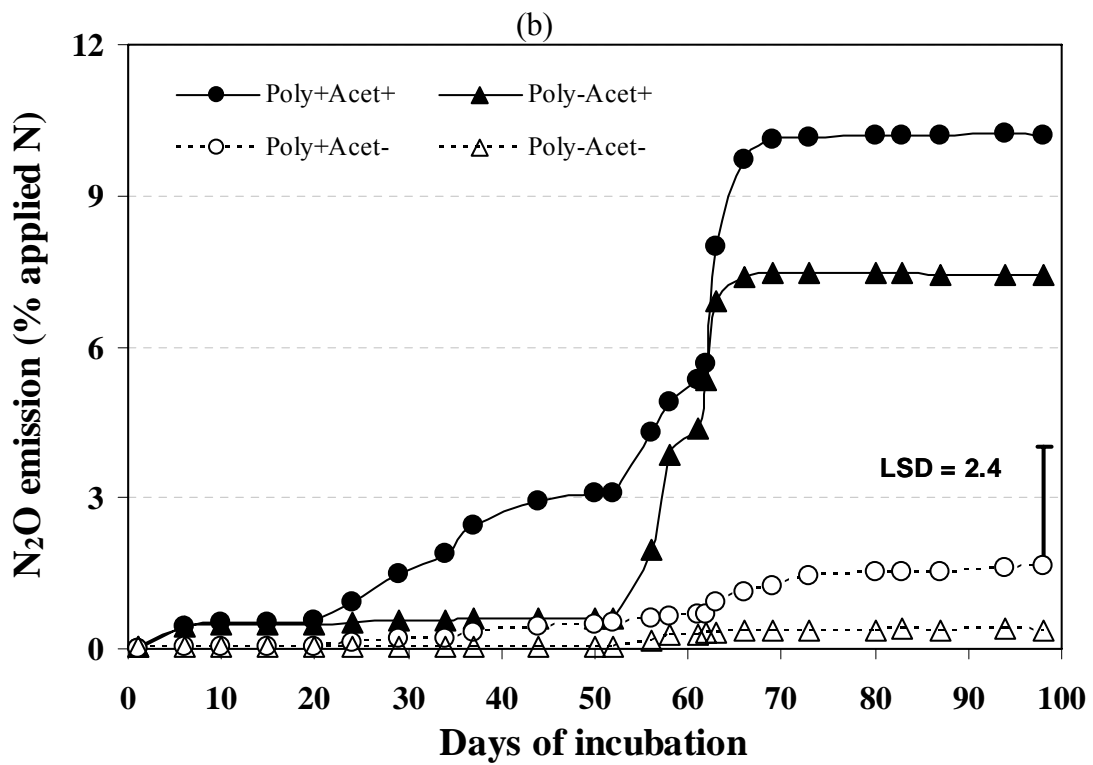
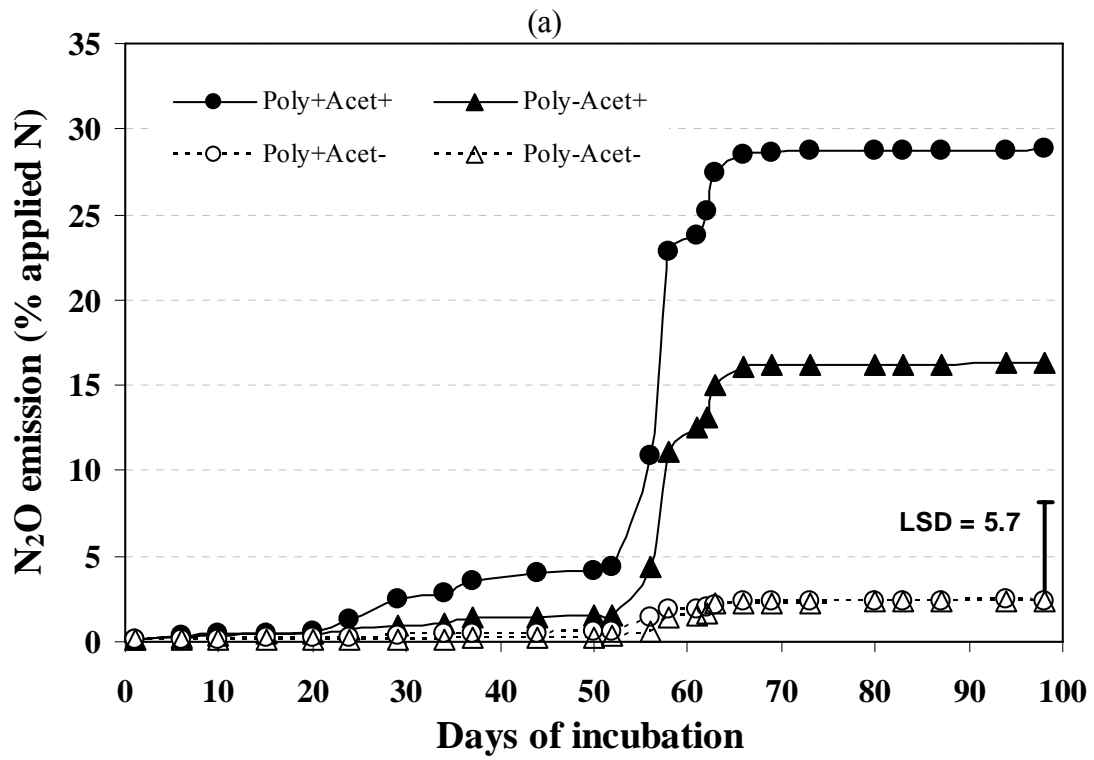
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717 Pu et al., Fig. 4

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