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Abstract: Batch experiments were performed to denitrify groundwater using sawdust as a carbon source at Marydale, South Africa. Alkalinity, pH, Electrical Conductivity, nitrate, nitrite, ammonia, SO₄²⁻, heterotrophic plate count (HPC), dissolved organic carbon (DOC), potassium and chloride was monitored. Two soil depths, 75 to 100cm and 165 to 200cm respectively, from the Marydale area were used as matrix material during denitrification based on contrasting chemical composition with respect to major ion composition and moisture to consider different denitrification rates for varying soil depths. Different N: C ratios were used to evaluate the denitrification efficiency and the least undesirable products e.g. elevated SO₄²⁻, H₂S and other reduced compounds. DOC is directly proportional to the N: C ratio used. Nitrite was produced for most of the treatments as incomplete denitrification occurred. The incubation periods were 28 and 43 days respectively. N:C ratios were 12.6:1, 24:1, 34:1 and 54:1. Longer incubation period and higher N: C ratio resulted in total removal of both nitrate and nitrite. The reaction was carbon limited for lower N: C ratios. The denitrification rate was proportional to the carbon availability at any time during the experiment. There was no significant difference in denitrification using heterogeneous and homogeneous particle sizes for sawdust. Soil depth of 75-100 cm displayed a greater denitrification rate than 165-200cm soil depth due to higher initial soil nitrate concentration. The method showed some specificity, as DOC, nitrite, nitrate, alkalinity and HPC were the only parameters that showed a change in concentration over the duration of the denitrification experiment under constant temperature and nitrogen gas atmosphere. DOC and HPC were unacceptable for domestic use, but methods such as boiling or chlorinating water can rid it of bacteria.

Response to Reviewers: Hi,

I have addressed all the comments in the decision letter. Please advise if any further revision may be necessary.

Thanks
Sumaya

1 **In Situ batch denitrification of nitrate rich**
2 **groundwater using sawdust as a carbon**
3 **source- Marydale, South Africa**

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7

8 **Abstract**

9 Batch experiments were performed to denitrify groundwater using sawdust as a carbon source at
10 Marydale, South Africa. Alkalinity, pH, Electrical Conductivity, nitrate, nitrite, ammonia, SO_4^{2-} ,
11 heterotrophic plate count (HPC), dissolved organic carbon (DOC), potassium and chloride was
12 monitored. Two soil depths, 75 to 100cm and 165 to 200cm respectively, from the Marydale area
13 were used as matrix material during denitrification based on contrasting chemical composition
14 with respect to major ion composition and moisture to consider different denitrification rates for
15 varying soil depths. Different N: C ratios were used to evaluate the denitrification efficiency and
16 the least undesirable products e.g. elevated SO_4^{2-} , H_2S and other reduced compounds. DOC is
17 directly proportional to the N: C ratio used. Nitrite was produced for most of the treatments as
18 incomplete denitrification occurred. The incubation periods were 28 and 43 days respectively. N:C
19 ratios were 12.6:1, 24:1, 34:1 and 54:1. Longer incubation period and higher N: C ratio resulted in
20 total removal of both nitrate and nitrite. The reaction was carbon limited for lower N: C ratios. The
21 denitrification rate was proportional to the carbon availability at any time during the experiment.
22 There was no significant difference in denitrification using heterogeneous and homogeneous
23 particle sizes for sawdust. Soil depth of 75-100 cm displayed a greater denitrification rate than
24 165-200cm soil depth due to higher initial soil nitrate concentration. The method showed some
25 specificity, as DOC, nitrite, nitrate, alkalinity and HPC were the only parameters that showed a
26 change in concentration over the duration of the denitrification experiment under constant
27 temperature and nitrogen gas atmosphere. DOC and HPC were unacceptable for domestic use, but
28 methods such as boiling or chlorinating water can rid it of bacteria.

29

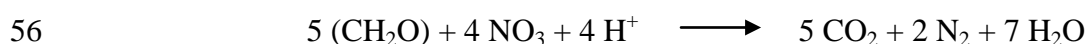
30 Introduction

31 In South Africa, high nitrate levels in groundwater is the single most important reason for
32 groundwater sources to be declared unfit for drinking, i.e. nitrate N exceeding 10 mg/L,
33 (World health organisation, 1998) (Marais, 1999). Although no statistics are available it
34 is known that infant methaemoglobinaemia occurs in southern Africa (Tredoux *et al.*
35 2000). The incidence of methaemoglobinaemia and the occurrence of high nitrate levels
36 in groundwater in Namibia and South Africa have triggered epidemiological studies for
37 investigating the effects of the sub-lethal levels of methaemoglobin on children (Tredoux
38 *et al.* 2005). The ideal drinking water class (“blue”, i.e. Class 0) has less than 6 mg/L
39 nitrate (plus nitrite) as N while the maximum allowable concentration is 20 mg/L as N in
40 South Africa (DWAF 1996). This is generally in agreement with the World Health
41 Organisation guidelines. However, in many areas of South Africa nitrate levels exceed
42 the completely unacceptable levels of 40 mg/L as N, and levels of 100 mg/L or even
43 greater than 200 mg/L are found in various places, (Tredoux and Talma 2006). Water
44 with nitrate exceeding 40 mg/L, belongs to the category of “dangerous” drinking water
45 quality (“purple”, i.e. Class IV) (DWAF *et al.* 1998). Such levels are an order of
46 magnitude higher than for example in the European Union where water with nitrate N
47 exceeding 5.5 mg/L has to be denitrified (Tredoux and Talma 2006).

48

49 In approximately 280 towns in South Africa, some of which have evaporation that
50 exceeds recharge or rainfall, groundwater is the sole source of water (Tredoux *et al.*
51 2005). This total dependence on the resource in Southern Africa increases the need to
52 have groundwater that is of a good enough quality to be consumed by people and animals
53 alike. Denitrification is part of the biogeochemical nitrogen cycle, which proceeds as
54 follows with the help of enzymes and bacteria:

55



57

58 However, in nature, the reagents in the forward reaction are not always present in
59 sufficient amounts to allow the reaction to proceed (Israel 2007). Literature references
60 document several successful field applications in many countries. Permeable Reactive
61 Barriers (PRB) have been tested to remove nitrate from groundwater and waste water
62 over a long period from bench scale to full-scale implementation plants (Blowes *et al.*
63 2000; Schipper and Vojvodic-Vukovic 2000; McRae *et al.* 1999; Liang *et al.* 2000; and
64 Robertson and Cherry 1995 & Robertson *et al.* 2000) in the USA, Canada, New Zealand
65 and many other countries. An important fact that the literature warns one of is that
66 successful implementation of any technology is based on a thorough understanding of the
67 site and its characteristics. A safe way to proceed is to first do bench scale treatability
68 tests, then column or small pilot scale tests and once proper site characterization is
69 completed, field scale testing can occur.

70

71 Many studies have shown successful denitrification. However, some of the sites were not
72 always as successful, and often scientists who have successfully denitrified their source
73 water through a permeable reactive barrier in one location have had failures in other areas
74 using similar techniques (Robertson *et al.* 2003). Successful denitrification of
75 groundwater has been practised in New Zealand for more than a decade, however, their
76 newest attempted denitrification of non point sources of nitrate from shallow groundwater
77 failed due to hydraulic constraints on the performance of their denitrification wall
78 (Schipper *et al.* 2004) . Hence the hydraulic properties of both the wall and aquifer are
79 integral properties to be monitored and assessed throughout the life of an operation
80 (Barkle *et al.* 2008). The aquifer hydraulic conductivity was reduced by a great
81 percentage during construction so that groundwater flow occurred under the wall rather
82 than through it (Barkle *et al.* 2008 and Schipper *et al.* 2004). Particle size within the
83 aquifer is an important parameter to evaluate, and more importantly so, the redistribution
84 thereof during construction. Repacking of particles caused the change in hydraulic
85 conductivity which rendered the wall ineffective (Barker *et al.* 2008). Laboratory models

86 based on data collected by Barkle *et al.* (2008) predicted an 87% reduction in hydraulic
87 conductivity, which should be an alarming outcome, and a definite indication of
88 potentially ineffective denitrification walls. It was predicted that, assuming proper
89 installation of a PRB, little change in porosity or hydraulic behaviour will occur at 10
90 years after installation (Li *et al.* 2005). However, 30-50 years after installation, fouling
91 may have occurred to such an extent that flow may bypass the wall by (Li *et al.* 2005).
92 Fouling occurs more quickly in aquifers that are more permeable, are more heterogeneous
93 or have greater major ion concentrations (Li *et al.* 2005).

94

95 The literature highlights problems and in some cases possible solutions. Some problems
96 and solutions are site specific e.g. sandy aquifers, coarse sand and gravel, and even deep
97 primary aquifers have been used as test sites. The knowledge that these treatment
98 technologies can operate successfully for decades as passive treatment systems with
99 minimal maintenance makes it very attractive for rural southern African areas. Given
100 extensive areas of water shortage, field denitrification plants in South Africa and its
101 neighbouring countries may prove to be invaluable in securing safer water for drinking.
102 There is currently no known technology in South Africa that is specifically removing
103 nitrate from groundwater, and methods that do remove nitrate (such as ion exchange or
104 reverse osmosis) from either surface or waste waters produce highly saline brines that are
105 often disposed of in potentially hazardous ways that may affect water quality.

106

107 The objectives of this paper is to a) denitrify soil and groundwater containing elevated
108 nitrate by adding a carbon and energy source , b) Vary incubation periods and compare
109 outcomes, c) Test whether nitrate concentrations can be reduced to within the ideal
110 drinking water category using sawdust as a carbon source and c) to test whether varying
111 the carbon to nitrogen ratio has an effect on the rate and efficiency of denitrification. It
112 follows on from initial treatability studies considering various carbon sources including
113 glucose, methanol, maize meal and sawdust (Israel 2007). Sawdust was selected due to its

114 slowly degradable nature and the literature references showing successful sawdust
115 treatment over long periods of time.

116

117 **The Study Area**

118 Marydale is situated in the Karoo area of the Northern Cape Province, between the towns
119 of Prieska and Groblershoop, with the nearest large town being Upington approximately
120 180 km north west of Marydale (Figure 1).

121 **Fig. 1: The study area, Marydale, Northern Cape, South Africa. Left:**
122 **location in South Africa. Upper right: topography (extracted from Google**
123 **Earth, 2006). Bottom right: distribution of boreholes in the area (Tredoux *et***
124 ***al.*, 2004)**

125

126 Figure 1 shows the topography and locality within South Africa. Marydale is a small
127 town with 2039 inhabitants that is solely dependent on groundwater (Department of
128 Water Affairs and Forestry 2003). Most of their boreholes were drilled in a riverbed and
129 adjacent flood plain with some boreholes located several kilometres away on
130 neighbouring farms.

131

132 A primary and a secondary aquifer are present in the Marydale area. The flow pattern
133 within the secondary aquifer is not well understood, however, average yields are lower
134 than in the primary aquifer. In summary, the primary aquifer has a higher yield and better
135 water quality than the secondary aquifer. The primary aquifer occurs in the low lying
136 areas between ridges, and is composed mainly of aeolian sedimentary deposits (sandstone
137 and silt) up to 12 m thick with bedrock composed of either quartzitic gneiss or granite.
138 Sand grains are sub rounded and grain size varies from coarse grained (with some cobble
139 sized grains) at depth to medium and fine grained at the surface. A large percentage of
140 fine-grained matrix material is present. Water levels measured in the area vary from about
141 5 m to approximately 25 m, with 10 m and shallower ascribed to the primary aquifer,
142 with deeper levels relating to the secondary aquifer. Denitrification was applied to the
143 aquifer material of the primary aquifer during the experiment.

144

145 **Experimental**

146
147 Soil sampling was done by digging a 2m by 2 m pit or profile. Samples were collected at
148 various depths based on the variation of soil colour and texture. Soil from a particular
149 depth with identical textural properties was thoroughly mixed to homogenize the sample.
150 A representative sample was then collected from the homogenized sample. The samples
151 were analysed for %carbon and % nitrogen in soil, total sulphur, exchangeable cations,
152 soluble cations and anions, inorganic carbon, pH, EC, heterotrophic plate count and
153 alkalinity (Figures 2 and 3). Figure 2 shows the profile dug at Marydale, South Africa,
154 while figure 3 and 4 show the carbon to nitrogen ratio along the depth profile as well as
155 the percentage moisture, pH, total sulphur with depth, and the soluble anions and cations
156 respectively. These were selected as parameters that would most likely affect
157 denitrification at specific depths of the profile.

158 **Fig. 2: Soil Profile dug in Marydale, South Africa**

159

160 **Fig. 3: Soil chemical and physical properties analysed before selecting soil**
161 **depths for experimental purposes**

162

163 Soil chemical and physical properties analysed for are represented in Figures 3 and 4. pH
164 ranges from about 8.1 to 9.5 along the profile with a general increase in pH with depth.
165 Total sulphur shows a dual peak at about 0.5 and 1.1m depth along the profile. The
166 sulphur peak coincides with a pH range of 8.9 to 9.2. Moisture percentage is rather low
167 for the top 0.5m of the profile, but increases with depth to a maximum of just under 14%.
168 It is believed that the calcareous layer may play a role in a perceived decreased rate of
169 evaporation or evapotranspiration with depth along the profile. The N: C ratio varies with
170 depth. This is indicative of the varying availability of carbon and nitrogen with depth
171 which would directly affect the rate of denitrification. Soluble cations and anions show
172 that sodium and chloride are the major ions in solution along the profile, with sulphate
173 and potassium levels being the second highest along the profile.

174

175 Fig. 4: Saturated paste extracts for soluble cations and anions of each soil
176 depth sampled

177
178 Saturated paste extracts as well as 1:5 soil: water solutions were analysed for cation and
179 anion compositions. Anions were analysed using an Inductively Coupled Plasma
180 technique, while cations were analysed using Atomic Adsorption Spectroscopy. Nitrate
181 and nitrite were determined using a nitrogen analyser. %C and %N were analysed using
182 an elemental analyser. Inorganic carbon was determined using a weight difference
183 technique. Initial weight of samples were determined, and weight after reacting with HCl.
184 The weight difference is then recorded, and the % inorganic carbon calculated.
185 Heterotrophic plate count was determine at every sampling time, by quantifying the
186 viable bacteria that were able to grow on a plate mixed with a nutrient agar, tryptone
187 glucose yeast agar, incubated for 48 hours at 35 degrees celcius. Grain size distribution
188 was analysed using sieve analyses to determine the percentage clay, silt, and sand sized
189 particles. Ten soil depths were analysed, with all having >90% sand sized particles which
190 classified them as sand. Silt and clay particles were below 5% in all samples.
191
192 pH was measured for both soil and water solutions. One soil profile was selected closest
193 to the borehole for which denitrification was tested as a representative of the aquifer
194 material where the water occurred.
195
196 Groundwater samples were collected at all boreholes in the area to get an understanding
197 of the water quality and composition in the area. Samples were collected by purging or
198 pumping the boreholes for at least 30 minutes prior to sampling. Sampling bottles were
199 thoroughly rinsed with water from the borehole before collecting the sample. Water
200 samples were analysed for major cations and anions, DOC, Fe, Mn, alkalinity, pH and EC
201 using standards methods. Measurements of EC, temperature, pH were taken in the field at
202 sample collection. Sawdust, the selected carbon source based on outcomes of previous
203 work (Israel, 2007 and Israel *et al.*, 2005) was also analysed for % carbon, % nitrogen, as
204 well as major cations and anions, Fe and Mn (Table 2).

205

206 **Table 1: Results of sawdust analyses**

207

208 Sawdust was analysed using ICP-OES. Samples were prepared by acid digestion.

209 Groundwater was sampled from the shallow primary aquifer; data shown in figure 4.

210

211 **Fig.5: Water type characterisation for Marydale, Northern Cape.**

212

213

214 N: C ratios were then calculated using the available data from soil, groundwater and

215 sawdust (carbon source) data. The volumes of sawdust, soil and groundwater required for

216 a certain N: C ratio was then determined. Soil and groundwater quantities were identical

217 in all samples, sawdust varied to accommodate the N: C calculated (Table 2).

218 **Table 2: Experimental details for laboratory batch incubation denitrification**
219 **experiments**

220

221 The calculation took into account the total carbon and nitrogen in soil, groundwater and

222 sawdust samples. This was used to determine the mass of sawdust required for the

223 reaction for specific N:C ratios. The only relation to field conditions was the actual

224 chemical composition; there was no relation between the volumes of reagents used and

225 field conditions. Water was added to saturate the soil.

226

227 Two depths of soil (75-100cm and 165-200cm) were selected for the experiment based on

228 the difference in EC, and anion and cation constituents, as well as the change in moisture

229 conditions with depth. Two separate experiments were run over 28 and 43 days

230 respectively. Samples were incubated in 50 ml bottles. A sample bottle was prepared for

231 every time of sample analysis. The objectives of the experiments were as follows:

232 Phase 1- to test or identify the effects of different N:C's on the rate of denitrification; and

233 Phase 2- to test the effects of larger N:C and extended incubation period.

234

235 Both experiments aimed to reduce nitrate and nitrite to within acceptable levels according

236 to domestic water requirements. N:C ratios that were used were 12.6:1, 24:1, 34:1 and

237 54:1, these are equivalent in the experiment to using 0.1g, 0.2g, 0.3g and 0.5g of sawdust

238 respectively. Samples were placed in an incubator set at a constant temperature of 23.4°C,
239 which was the field temperature of the groundwater at the borehole sampled in Marydale.
240 Temperature was measured by thermometer as well as a pH meter. Nitrogen gas was
241 passed through the incubator to maintain an oxygen free environment. Samples were then
242 removed at sampling times $t = 0, 0.125, 0.25, 0.5, 1, 2, 4, 7, 10, 14$ and 28 days for the
243 first experiment, and $t = 0, 3, 7, 10, 14, 21, 29$ and 43 days for the second experiment
244 (Table 2). The times were selected based on results from prior experimenting (Israel,
245 2007) which showed that during weekly sampling, major changes were possibly missed
246 during the first week of the experiment. Samples were analysed for heterotrophic plate
247 counts, nitrate, nitrite, EC, pH, alkalinity, sulphate, chloride, dissolved organic carbon
248 and ammonia. Samples collected at $t = 0, 10$ and 28 (for the first batch of samples) and t
249 $= 0, 10$ and 43 (for the second batch of samples) were analysed for Fe, Mn, major cations
250 and anions as well as the above parameters. Statistical comparisons were made between
251 the datasets and within the second experiment as all samples were prepared in triplicate.
252 The student t-test as well as box and whisker's which records the min, max, mean and the
253 level of significant difference between two sampling points were used. Correlation
254 matrices were done to assess the relation between the variables in the experiment.

255 **Results and Discussion**

256 **Soil, groundwater and sawdust characteristics**

257 Soil and groundwater was analysed and later mixed, and different N: C ratios of sawdust
258 added. Table 3 shows the results of the initial mixing of the soil, groundwater and
259 sawdust at the various ratios (i.e. 12.6:1 or 0.1g sawdust, 24:1 or 0.2g sawdust, 34:1 or
260 0.3g sawdust respectively).

261 Table 3: Results of mixing sawdust, groundwater and soil for laboratory 262 testing of sawdust as a carbon source for denitrification
--

263
264
265 This was compared to drinking water standards set (DWAF, 1993 &1996) for South
266 Africa and in some cases to the (WHO, 1998) guidelines.

267

268 The shaded values in table 3 represent the initial concentrations of nitrogen species in
269 solution as well as the dissolved organic carbon (DOC), pH and heterotrophic plate count
270 which were selected as indicator parameters for this experiment. Note that there was no
271 ammonia and nitrite present at the start of the experiment. Nitrate concentrations were
272 above the maximum allowable of by DWAF, 1993 & 1996 of 20mg/L drinking water
273 standards and the WHO, 1998 level of 10mg/L maximum allowable.

274

275 The pH of the soil, groundwater and sawdust mixture is above that of the optimum range
276 of 7-8 for denitrification as described in the literature. The pH values measured in soils
277 were between 9 and 9.5. Initial dissolved organic carbon is greater for larger carbon to
278 nitrogen ratios, which indicates that there is a direct relationship with the amount of
279 sawdust in the mixture. Nitrate concentrations are higher in shallower soil groundwater
280 mixtures. Heterotrophic plate count is greater in the 165-200cm-soil depth mixture.
281 Sodium and potassium is greater in the shallower soil while Ca and magnesium
282 concentrations are greater in deeper soil samples.

283

284 The analyses of sawdust used in the experiment showed that it contained 0.25% nitrogen
285 and 53.25% carbon. These percentages were incorporated into an equation to find the
286 mass of sawdust to use for specific N: C ratios. Table 2 shows the parameters and their
287 concentrations for sawdust used during the denitrification experiment. Trace metals like
288 Fe, Mn, Cu, Mo, Co and Zn act as enhancers for denitrification as bacteria and enzymes
289 require them, (Labbé *et al.* 2003 and Lee 1996).

290

291 **Phase 1 (28 day experiment)**

292 Nitrate levels were plotted for each treatment and for both soil depths for the entire
293 period, for which laboratory testing took place. The nitrate concentrations decreased with

294 time for both 75-100cm and 165-200cm soil depths with slightly varying rates for the
295 different N:C ratios.

296

297 Figure 6 shows that after 28 days of incubation at 23°C (the field temperature of the
298 water) two of the treatments show a decrease in nitrate concentration to below 10 mg/L,
299 the acceptable level, for the 75-100cm deep soil sample and groundwater mixture.

300

301 Fig. 6: Nitrate, DOC, nitrite and alkalinity (on the right hand y-axis)
302 concentrations for the duration of the laboratory testing for the 75-100 cm
303 deep soils, units mg/L.

304

305

306 All the treatments reduce the nitrate concentration to below the maximum allowable
307 within the period of laboratory testing. A similar graph was plotted for the 165-200cm
308 soil depth (Figure 7) to evaluate whether there was any distinct difference between the
309 results.

310

311 Fig. 7: Nitrate, DOC, nitrite and alkalinity (on the right hand y-axis)
312 concentrations for the duration of the laboratory testing for the 165-200 cm
313 deep soils, units mg/L.

314

315

316 The 165-200cm deep soil and groundwater mixture (Figure 8) also shows that the 24:1
317 and 34:1 N: C ratios reach a nitrate concentration within the acceptable levels for
318 drinking water (i.e. 6 mg/L as N). The 75-100cm depth of soil and groundwater mixture
319 seems to be more effective than the 165-200cm in reducing the nitrate concentration. The
320 slopes of the curves show an earlier decline for the 75-100cm than in the 165-200cm deep
321 soil for all treatments.

322

323 The 24:1 and 34:1 treatments resulted in a nitrate concentration within acceptable levels
324 i.e. below 10 mg/L for both soil depths. Dissolved organic carbon, nitrate, nitrite and
325 nitrate plus nitrite are represented in figure 6 and 7 for both soil depths as well as all N: C
326 ratios used. Dissolved organic carbon shows the amount of carbon available in the
327 dissolved phase to take part in the denitrification reaction at any given time during the

328 experiment. Nitrate and nitrite were plotted to see to what extent denitrification
329 proceeded.

330

331 Although denitrification occurred and nitrate concentration reached acceptable levels, it
332 was found that for most treatments, nitrite was being produced (Figures 6 and 7). It
333 increased as the nitrate concentration decreased. This was an indication of an incomplete
334 denitrification reaction, and that nitrate was being converted to nitrite. The levels of
335 nitrite produced were above the maximum allowable concentration for nitrite which
336 according to the world health organization guideline values is 1mg/L as nitrite is ten
337 times more potent than nitrate on a molar level with respect to haemoglobin, (WHO,
338 1998). This nitrite production may have been caused by competing effects between
339 nitrifying bacteria present and denitrifying bacteria present.

340

341 The 75-100cm soil treated samples (Figure 6) showed a slightly different result from
342 figure 7. Although the graph shows an increase in the nitrate concentration for the last
343 sample, it also shows that nitrite was totally removed from the system. This production of
344 nitrite and incomplete denitrification reaction could be owed to the limited availability of
345 carbon. The dissolved organic carbon shows that availability of carbon fluctuates with
346 time; denitrification may have been limited by carbon availability as sawdust is a slowly
347 releasing carbon substrate. Sawdust is a slowly degrading carbon source and would most
348 likely initially release the soluble organic constituents of the sawdust (i.e. tannic acids
349 etc.), followed by the less readily available organic carbon portion, a similar statement is
350 made by Robertson and Cherry 1995 and Robertson *et al.* 2000 & 2003.

351

352 The distinct difference between the two treatments above is that the nitrate + nitrite
353 concentration decreases for 75-100 cm deep soil, while it remains almost constant for
354 165-200 cm deep soil. It is evident that the dissolved organic carbon in Figure 7 (0.3 g
355 sawdust treatment) decreases after t=14 days, while it shows a negligible decrease for

356 Figure 6 (0.3 g sawdust treatment). This again brings to fore the idea of the reaction being
357 carbon limited. Since nitrite was produced in all treatments i.e. 12.6:1, 24:1, and 34:1 for
358 both soil depths, nitrite vs. nitrate was plotted to see what the relationship between the
359 two parameters was (Figure 8).

360

361 Figure 8: Nitrate vs. nitrite, times - symbols in legend. Arrows - time scale.
362 Sequence of time - top left to bottom right, then right to left. Time in the
363 legend is in hours

364

365 It is evident from Figure 8 that nitrite increases or is produced while nitrate is reduced
366 during the reaction. Nitrite has been reported in the literature as an intermediate of
367 denitrification (Mateju, 1989; Robertson and Cherry, 1999). The points in the upper left
368 area of the graph represent the starting point of the experiment as well as the untreated
369 samples' results. One treatment in the 75-100cm soil shows the reaction proceeding
370 further and removing nitrite from the system. The circled point on the graph represents
371 the ideal outcome of the experiment. Where most nitrate is reduced i.e. nitrate levels
372 within the acceptable range for drinking water, and nitrite levels approaching zero.

373

374 The results of the samples after the 28 day denitrification experiment follow. Table 4
375 represents the results of samples analysed after 28 days of incubation relating to the first
376 row of table 1 in the experimental section.

377

378 Table 4: chemistry data for samples analysed after the 28 day laboratory
379 denitrification experiment

380

381

382 Incubation was done at 23°C, the groundwater temperature measured in the field. The
383 shading indicates values that exceed the target water quality range for domestic use set
384 out by DWAF. Where no shading is present, values are within the acceptable levels for
385 potable use.

386

387 Nitrate levels decreased to within the target range (0-6mg/L nitrate as N - DWAF, 1993)
388 for the 1:24 and 1:34 nitrogen to carbon ratios. Nitrite levels in certain of the treatments

389 have increased to above the maximum allowable levels. Only the 1:34 treatment using the
390 75-100cm-soil depth and phase II of the experiment shows the nitrite levels approaching
391 and reaching zero. This raised questions about extending the time of incubation and
392 increasing the amount of sawdust (carbon source) in the reaction.

393

394 The heterotrophic plate count was measured throughout the experiment to evaluate how
395 quickly the denitrifiers establish their colonies and whether there is a noticeable die-off in
396 the curve. A lag phase is present from $t=0$ to $t=6$. Major growth occurs between $t=6$ and
397 $t=24$. After $t=24$, growth slows down and most of the treatments maintain similar
398 numbers of colonies. The untreated and 0.1g sawdust treated in the 165-200cm show
399 growth between $t=6$ and $t=12$ and then between $t=24$ and $t=48$. After $t=48$ most of the
400 points occur within a band of values. This shows that initial and final heterotrophic plate
401 count data are similar for all treatments.

402

403 Establishment and growth of heterotrophic bacteria is an indication that the conditions in
404 the system are indeed suitable for their growth and for denitrification to be facilitated by
405 indigenous microbial colonies.

406

407 **Phase 2 (43 day experiment)**

408

409 Further tests done for 0.3g and 0.5g treatments over a longer period showed that all
410 nitrate and nitrite could be removed from the system and produced gaseous products.

411 Elevated levels of DOC and alkalinity produced followed similar trends for all treatments.

412 To verify the results produced by the 0.3g treatments it was included again in phase 2. To
413 evaluate the results of a longer period of incubation and a greater N:C, 0.5g or 54:1
414 sawdust treatments were also used. The results show that 0.3g and 0.5g treatments
415 (Figures 9 and 10 respectively) show more favourable reactions over the longer period
416 than that of the N: C ratios used before.

417

418 Figure 9: 0.3g treatments where a, b, and c represents the concentrations
419 of nitrate, nitrite, DOC, and Alkalinity/10 over 43 days of incubation under
420 N₂ (g) atmosphere for 40mL groundwater, heterogeneous sawdust
421 particles and 10g soil mixtures, while d represents a similar mixture with
422 sawdust particles that are fine and homogenous.

423
424 Fig. 10: nitrate, nitrite, DOC and alkalinity/10 for 0.5g treatments where a,
425 b, and c represents 43 days of incubation under N₂ (g) atmosphere for
426 40mL groundwater, heterogeneous sawdust particles and 10g soil
427 mixtures, while d represents a similar mixture with sawdust particles that
428 are fine and homogenous.

429
430 No measurable concentrations of nitrite occurred toward the end of Phase 2, this was an
431 indication of denitrification being complete. Gas production occurred during the
432 experiment. This could be either CO₂ (g) and possibly N₂ (g) production, although these
433 parameters were not analysed for.

434

435 Standard deviations were very small between replicate samples, hence the errors were
436 within acceptable limits statistically. DOC is the only parameter that shows large errors
437 (see Figures 11 and 12); this is due to the slow intermittent release of carbon from the
438 sawdust, this release is independent of the rate of reaction.

439

440 Figure 11: Box and whisker plots for indicator parameters analysed in
441 triplicate for 43 day incubation experiments using 0.3g sawdust treated
442 samples.

443

444 Figure 12: Box and whisker plots for 43 day incubation experiments of
445 0.5g sawdust treated samples of soil and groundwater

446

447

448 The reaction rate is accelerated when more carbon is used. Phase 2 shows total removal
449 of nitrate by day 10 in the 0.3g treatment and by day 7 in the 0.5g treatment. Subsequent
450 production of nitrate could be conversion from nitrite to nitrate in solution. Eventual
451 removal of both nitrate and nitrite is achieved within the incubation period of 43 days. A
452 steady increase in alkalinity is seen in both figures 11 and 12 as in phase 1, which is an
453 indication of biological activity within the samples.

454

455 Denitrification proceeded successfully without the addition of microbes or bacteria to any
456 of the samples. Electrical conductivity tends to increase as pH decreases and alkalinity
457 increases.

458

459 **Comparison of Phase 1 and 2 data**

460

461 Experimental Phases 1 and 2 were performed months apart, hence it had to be assessed
462 whether the results were comparable. Identical experimental settings were used in each
463 experimental procedure, however, only 34:1 or 0.3g sawdust treatments were identical
464 between the two experiments. Comparison of the data was plotted to evaluate the
465 relationship between the data and to decide whether the method was indeed repeatable.

466 Parameters were selected as indicator parameters of denitrification; these included
467 nitrogen species nitrate, nitrate and ammonia, dissolved organic carbon, alkalinity, pH,
468 EC and heterotrophic plate count. The data sets from phase 1 and 2 were then plotted for
469 nitrate-N, nitrite-N and alkalinity vs. time (figures 13, 14, and 15). This shows that data
470 from phase 2 was comparable to that in phase 1. The plots for specific variables follow
471 similar trends and have concentration ranges in the same order of magnitude for specific
472 times.

473 Fig. 13: Nitrate-N for the duration of incubation experiments. The + sign
474 represents 0.3g sawdust treatment containing 40mL groundwater, 10g soil
475 (75-100cm layer) incubated for 30 days (experiment 3), the triangle
476 represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer)
477 incubated for 43 days and done in triplicate.

478

479 Figure 14: Nitrite-N for the duration of incubation. + represents 0.3g
480 sawdust, 40mL groundwater, 10g soil(75-100cm layer) and 30 days
481 incubation in experiment 3, while the triangle represents 0.3g sawdust,
482 40mL groundwater, 10g soil (75-100cm layer) and 43 days incubation in
483 experiment 4 done in triplicate.

484

485 Figure 15: Alkalinity as CaCO_3 mg/L for the duration of incubation. The +
486 sign represents 0.3g sawdust treatment containing 40mL groundwater,
487 10g soil (75-100cm layer) incubated for 30 days (experiment 3), the
488 triangle represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm
489 layer) incubated for 43 days and done in triplicate.

490

491 **Conclusions**

492 The following conclusions were drawn from the results:

493

494 For 12.6:1 N: C ratio using sawdust:

495

496 • This N:C ratio did not reduce the nitrate concentration to below the acceptable
497 levels required for potability

498 • Little available carbon for denitrification led to production of nitrite and hence
499 incomplete denitrification

500 • Heterotrophic plate count was independent of the N:C ratio, but rather a function
501 of time

502

503 For 24:1 N: C ratio using sawdust:

504 • Nitrate levels were reduced to within acceptable levels for drinking

505 • Nitrite production occurred which was indicative of incomplete denitrification

506 • Nitrite levels persisted until the end of the batch experiment

507 • Alkalinity was inversely proportional to nitrate levels

508 • DOC was variable throughout the experiment with intermittent release and
509 consumption with time

510 • Heterotrophic plate count was a function of time rather than N:C ratio

511

512 For 34:1 N: C ratio using sawdust:

513 • Nitrate levels were reduced to within acceptable levels for drinking

514 • Nitrite production occurred which was indicative of incomplete denitrification

515 • A decrease in nitrite levels toward the end for 75-100cm deep soil showed that
516 time and N:C have a distinct controlling effect on the denitrification rate

517

518 Overall Conclusions:

519

520 • The NO_3^- concentration can be reduced to below the acceptable limit for potable
521 water

522 • Presence of NO_2^- is an indication of incomplete denitrification as a result of
523 limited carbon availability and limited incubation period

524 • DOC is directly proportional to the carbon to nitrogen ratios

525 • Using larger N:C ratios of sawdust as well as longer period of incubation
526 successfully removed nitrate and nitrite for both 0.5g treatment and 0.3g
527 treatments within the 43 day experiment

528 • The rate of nitrate removal is proportional or affected by the amount of sawdust
529 (carbon source) available during the reaction time

530 • There was no significant difference between denitrification using heterogeneous
531 particle size and homogenous fine particles of sawdust

532 • 75-100cm soil displayed greater denitrification rates than the 165-200cm due to
533 higher initial nitrate concentration in the particular soil depth.

534 • The method was specific in that DOC, nitrite, nitrate, alkalinity, pH and the
535 heterotrophic plate count were the only parameters affected by the denitrification
536 experiment, major cations and anions showed no notable changes.

537 • Nitrate and nitrite levels reach zero, however, DOC and heterotrophic plate count
538 do not comply with acceptable levels for drinking water for most treatments, but
539 secondary treatment methods such as boiling or chlorinating the water can rid it
540 of bacteria.

541 • The experiment is reproducible for constant temperature, under nitrogen gas
542 atmosphere, where, high nitrate containing groundwater and soil is mixed with a
543 slowly degradable carbon source.

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545

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Table 1: Soil grain size distribution along soil profile

Depth	Soil depth (cm)	Sample	%clay	%silt	%sand	classification
0-15 cm	15	# 0	2.6	3	94.4	Sa
15-30 cm	30	# 1	3.2	1.8	95	Sa
30-45 cm	45	# 2	3	2.2	94.8	Sa
45- 55cm	55	# 3	2.2	1	96.8	Sa
55- 75cm	75	# 4	1.8	3.2	95	Sa
75- 100cm	100	# 5	3.2	5.6	91.4	Sa
100-115cm	115	# 6	2.4	3	94.6	Sa
115-135cm	135	# 7	0.4	2.6	97	Sa
135-165cm	165	# 8	0.4	2.2	97.4	Sa
165-200cm	200	# 9	0.4	2	97.6	Sa

Table 2: Results of sawdust analyses

	Carbon %	N	P	K %	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	mg/kg											
Sawdust	53.25	0.25	0.03	0.08	0.10	0.04	124	20	58	1	10	5

Table 3: Experimental details for laboratory batch incubation denitrification experiments

Soil Depth	Soil (g)	Groundwater	Sawdust (g)	Repetitions and sampling series
75-100 cm and 165- 200 cm	10 g	40 ml	0, 0.1, 0.2, 0.3	t = 0, 0.125, 0.25, 0.5, 1, 2, 4,7, 10, 14, and 28 days
75-100 cm	10 g	40 ml	0.3 and 0.5	t = 0, 3, 7, 10, 14, 21, 29, and 43 days triplicate samples + one with identical mass but finer sawdust particles

Table 4: Results of mixing sawdust, groundwater and soil for laboratory testing of sawdust as a carbon source for denitrification

Treatment	untreated	1:12.6	1:24	1:34	untreated	1:12.6	1:24	1:34
Soil Depth	75-100				165-200			
Time (hrs)	Initial sample analyses for mixing of sawdust, groundwater and soil							
Potassium as K mg/L	74	76	79	76	59	60	68	61
Sodium as Na mg/L	372	394	409	398	308	308	366	316
Calcium as Ca mg/L	14	18	16	18	26	25	13	25
Magnesium as Mg mg/L	13	16	15	17	21	21	12	21
Ammonia as N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Sulphate as SO ₄ mg/L	150	156	165	149	114	131	146	136
Chloride as Cl mg/L	285	303	310	313	238	238	256	238
Alkalinity as CaCO ₃ mg/L	322	345	346	345	328	328	342	328
Nitrate plus nitrite as N mg/L	23.29	25.00	24.76	24.51	20.85	21.10	21.34	20.61
Nitrate as N mg/l	23.29	25.00	24.76	24.51	20.85	21.10	21.34	20.61
Nitrite as N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Iron as Fe mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Manganese as Mn mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Silica as Si mg/L	23.4	24.2	26.6	27.3	30.5	30.5	28.9	30.8
D OC mg/L	2.6	10.7	13.4	17.2	3.2	7.7	18.3	27.8
Conductivity mS/m (25°C)	200	215	218	215	180	180	190	180
pH (Lab) (20°C)	8.4	8.4	8.4	8.3	8.2	8.2	8.4	8.0
TDS (Calc) mg/L	1280	1376	1395	1376	1152	1152	1216	1152
Hardness as CaCO ₃ mg/L	88	111	102	115	151	149	82	149
HPC	2115	2165	2040	2620	5850	4600	4650	6250

Table 5: Chemistry data for samples analysed after the 28 day laboratory denitrification experiment

Treatment	Untreated	1:12.6	1:24	1:34	Untreated	1:12.6	1:24	1:34
Soil Depth	75-100				165-200			
Time (hrs)	Results after 28 day laboratory denitrification							
Potassium as K mg/L	86	91	90	89	72	72	72	74
Sodium as Na mg/L	392	405	400	403	301	321	309	313
Calcium as Ca mg/L	13	19	25	24	23	25	29	33
Magnesium as Mg mg/L	12	18	22	22	20	22	27	28
Ammonia as N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Sulphate as SO ₄ mg/L	150	158	172	191	120	145	142	147
Chloride as Cl mg/L	304	306	300	302	248	244	235	237
Alkalinity as CaCO ₃ mg/L	335	403	445	491	322	357	393	415
Nitrate as N mg/l	24.0	15.4	2.7	3.5	20.0	18.6	4.0	2.6
Nitrite as N mg/L	<0.1	4.6	12.3	0.5	<0.1	0.44	9.0	10.4
Silica as Si mg/L	20	28	29	29	35	35	44	45
DOC mg/L	2.6	4.1	7.9	15.1	2.2	3.6	12.8	13.1
Conductivity mS/m (25°C)	211	217	220	221	168	183	180	185
pH (Lab) (20°C)	8.4	8.1	8	8.2	8.3	8	7.9	7.9
TD S (Calc) mg/L	1350	1389	1408	1414	1075	1171	1152	1184
HPC (colonies per millilitre)	800000	5000000	8000000	7000000	620000	1500000	3500000	5000000

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Figure 10: Nitrate, nitrite, DOC and alkalinity/10 concentrations over 43 day incubation experiments using 0.5 g treatments with a, b, and c representing 40 mL groundwater, 10 g soil, and 0.5 g heterogeneous sawdust particles, while d has a similar mixture with sawdust particles being homogeneous.

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Figure 1

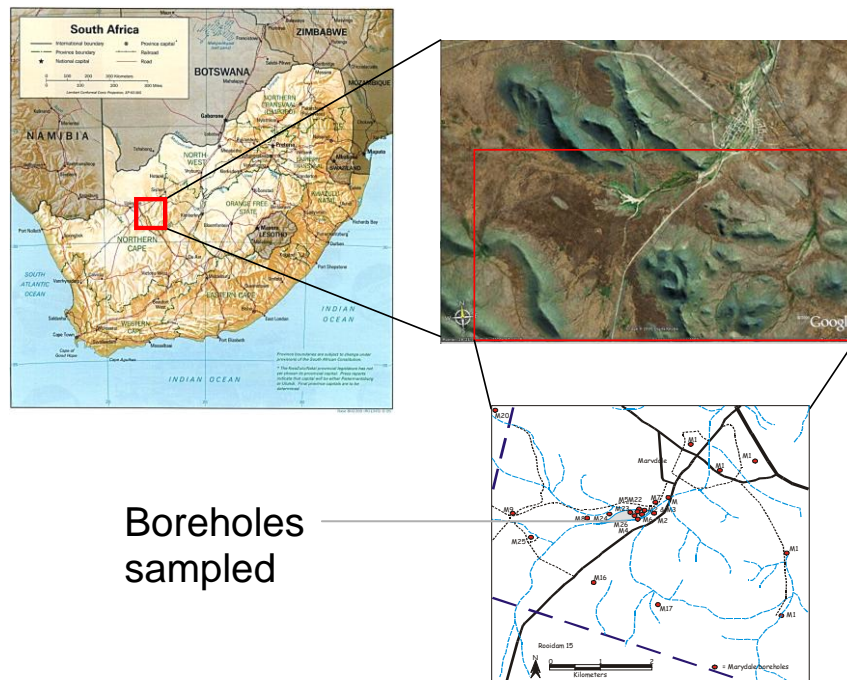


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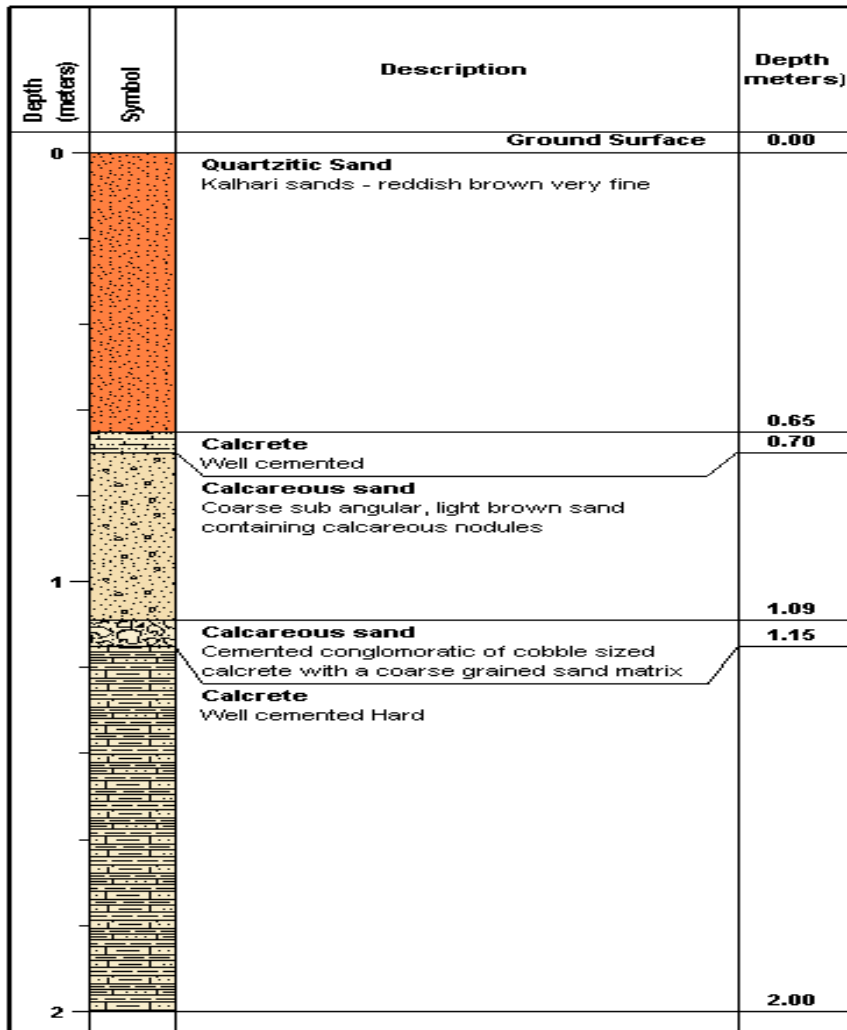


Figure 2: Soil profile dug in Marydale, South Africa

Figure 3

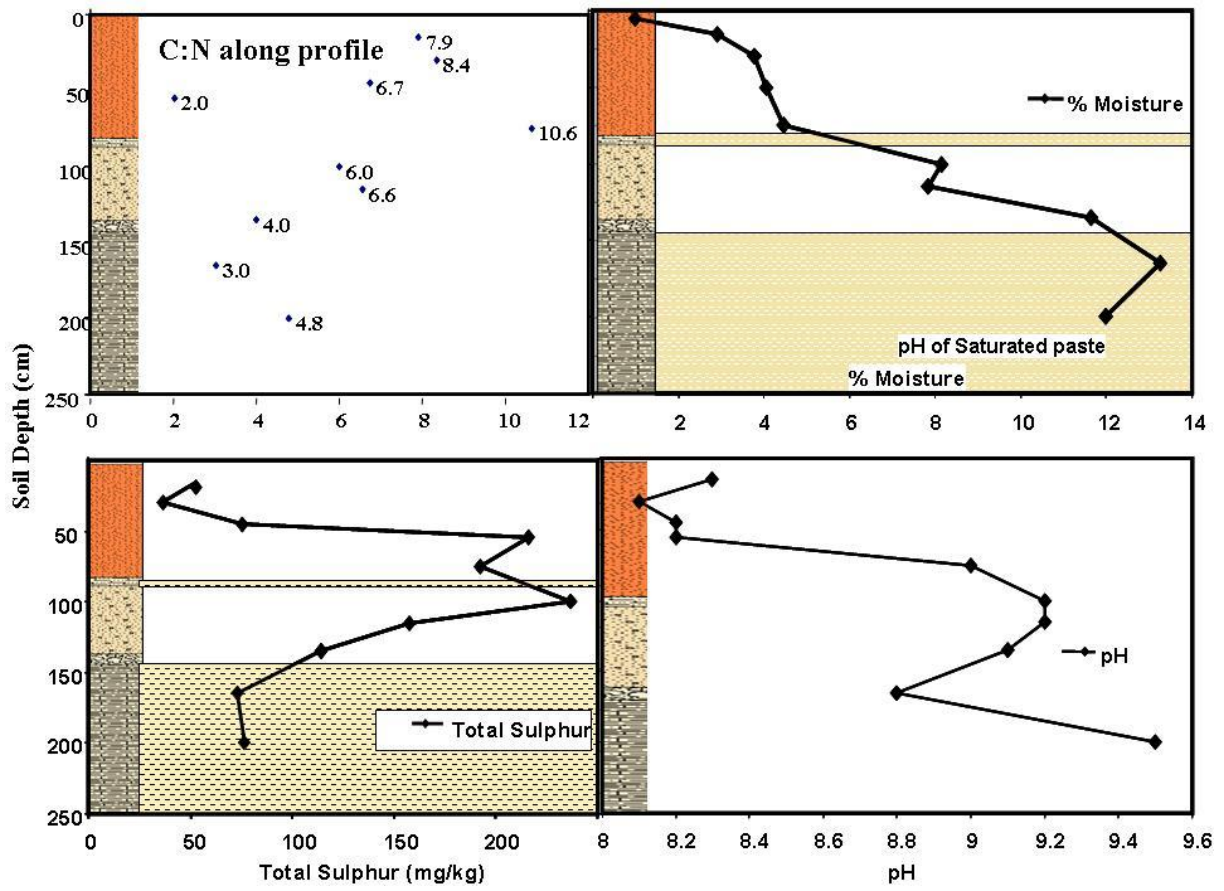


Figure 3: Soil chemical and physical properties analysed before selecting soil depths for experimental purposes.

Figure 4

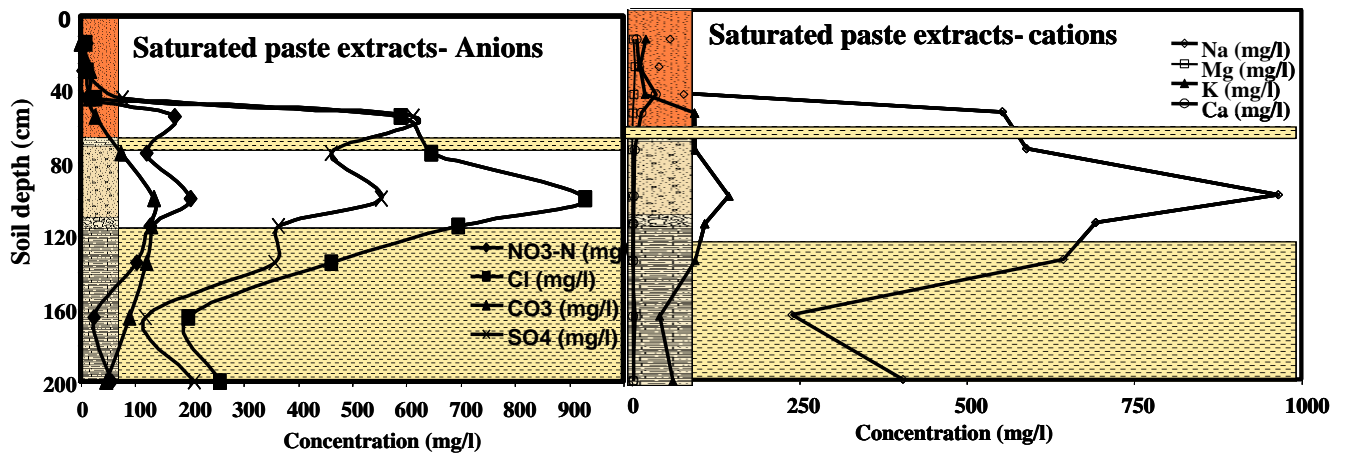


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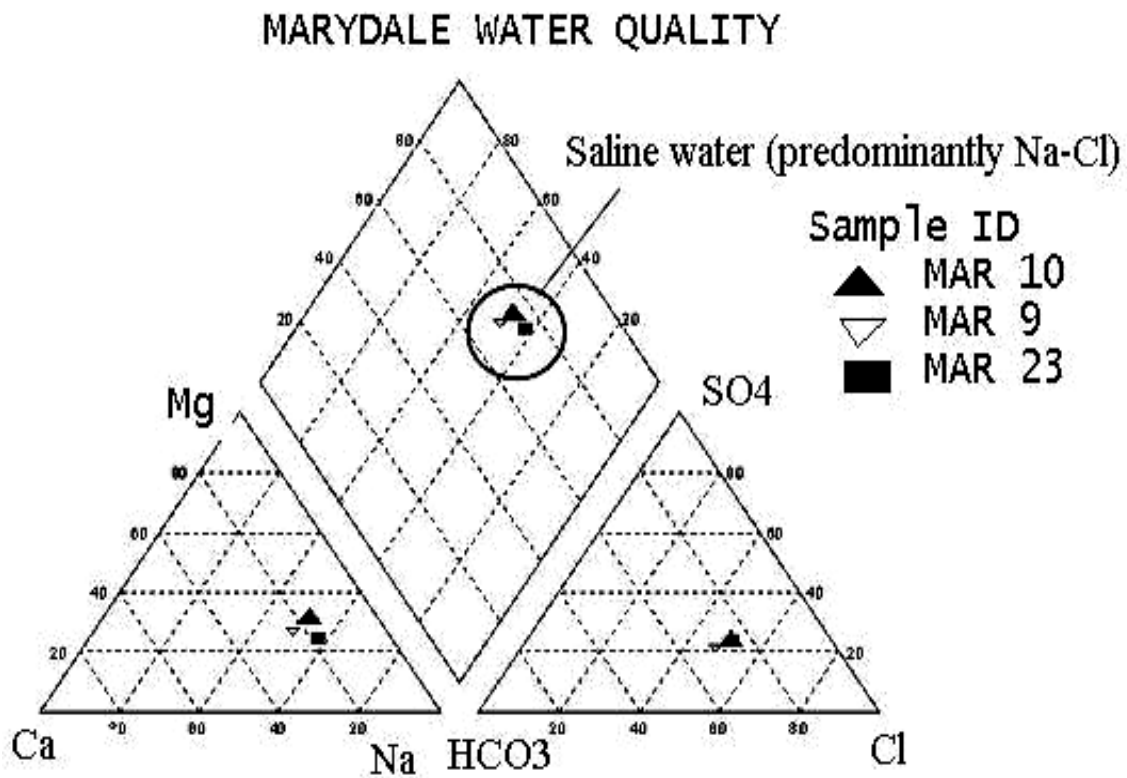


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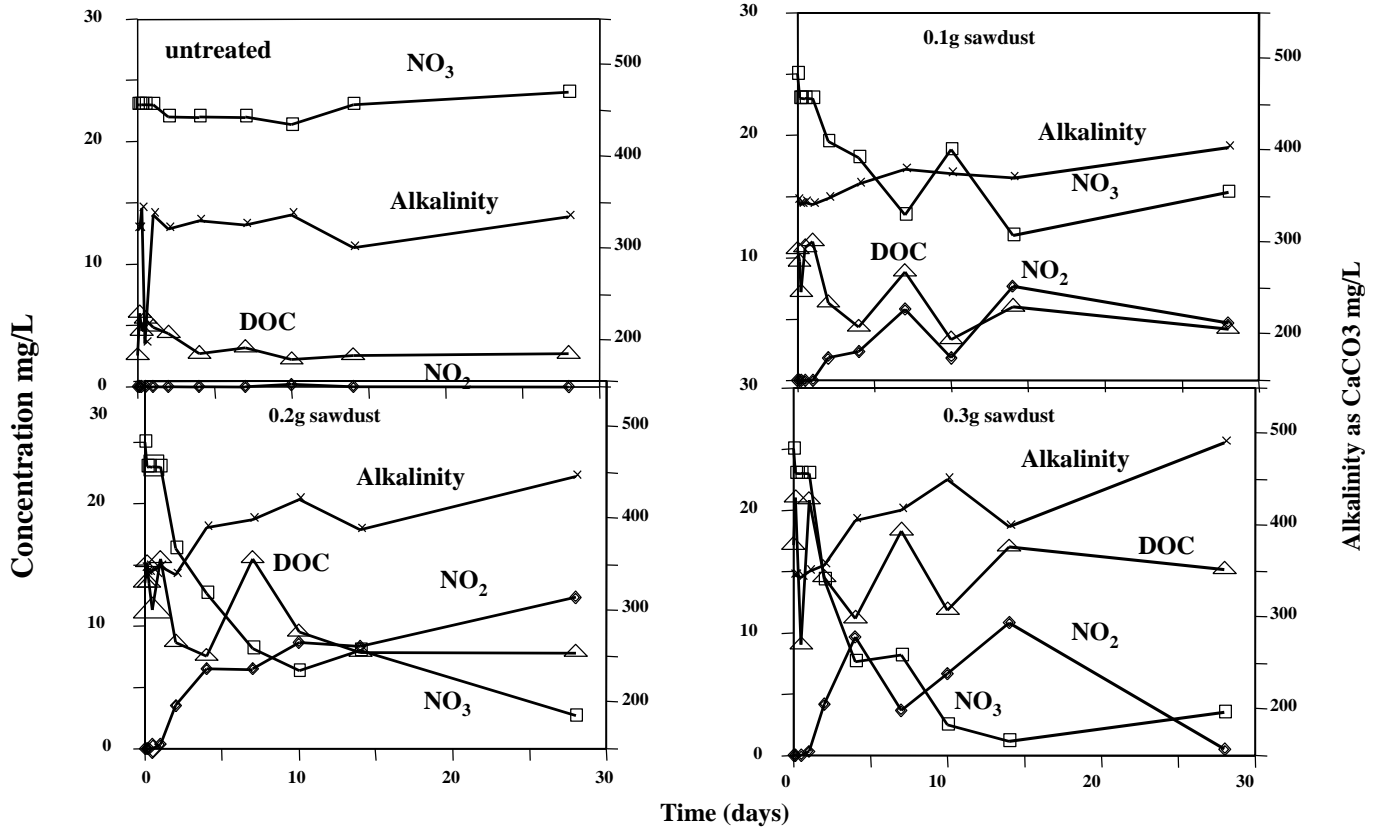


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Figure 7

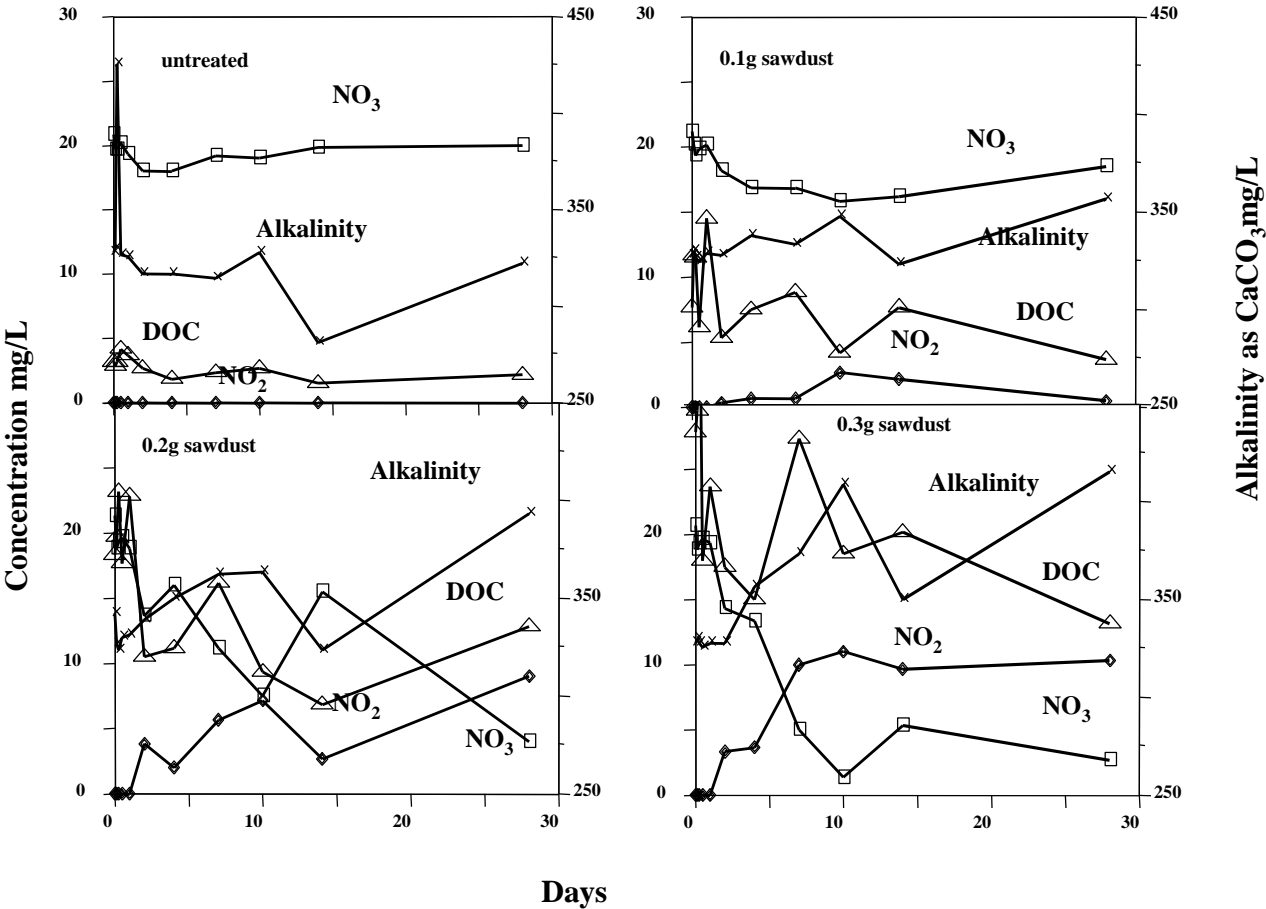


Figure 7: Nitrate, DOC, nitrite and alkalinity (X- on the right hand y-axis) concentrations for the duration of the laboratory testing for the 165-200cm deep soils.

Figure 8

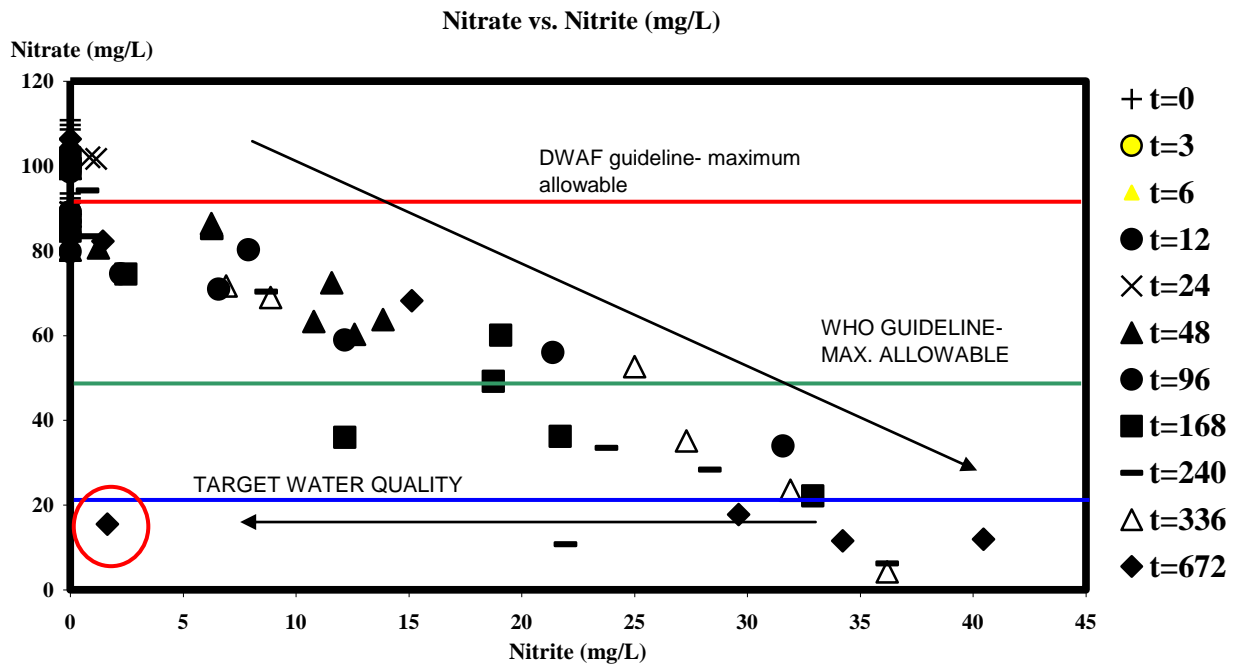


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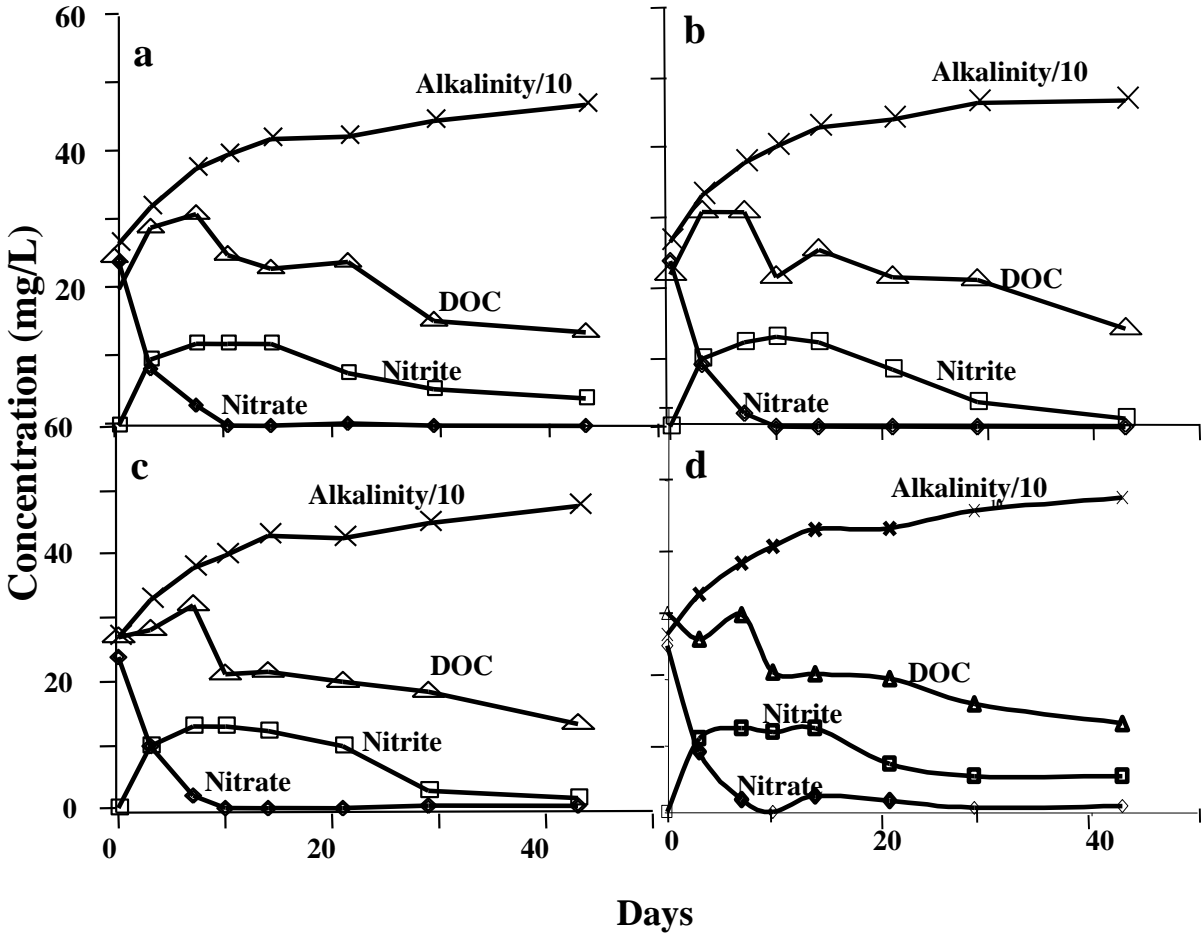


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Figure 10

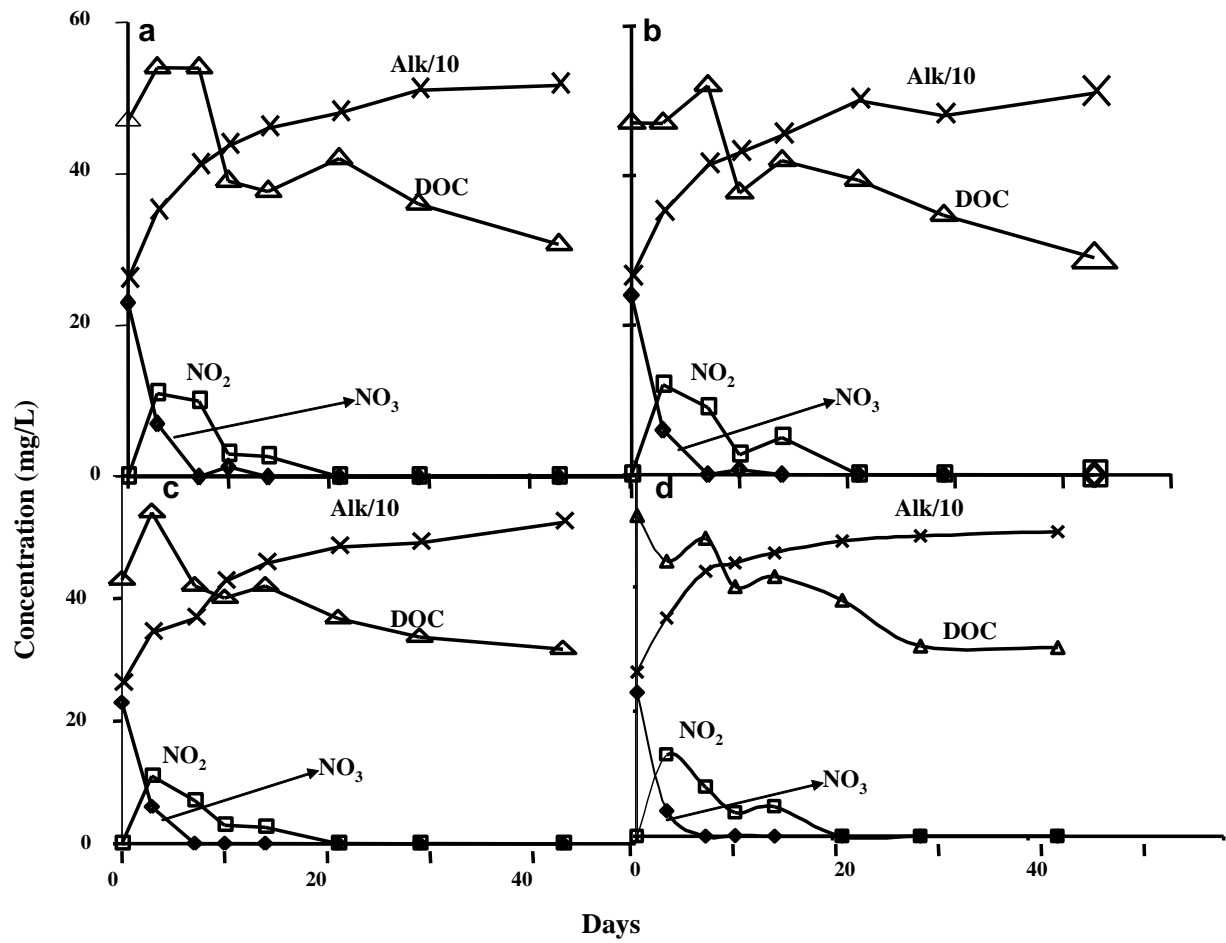


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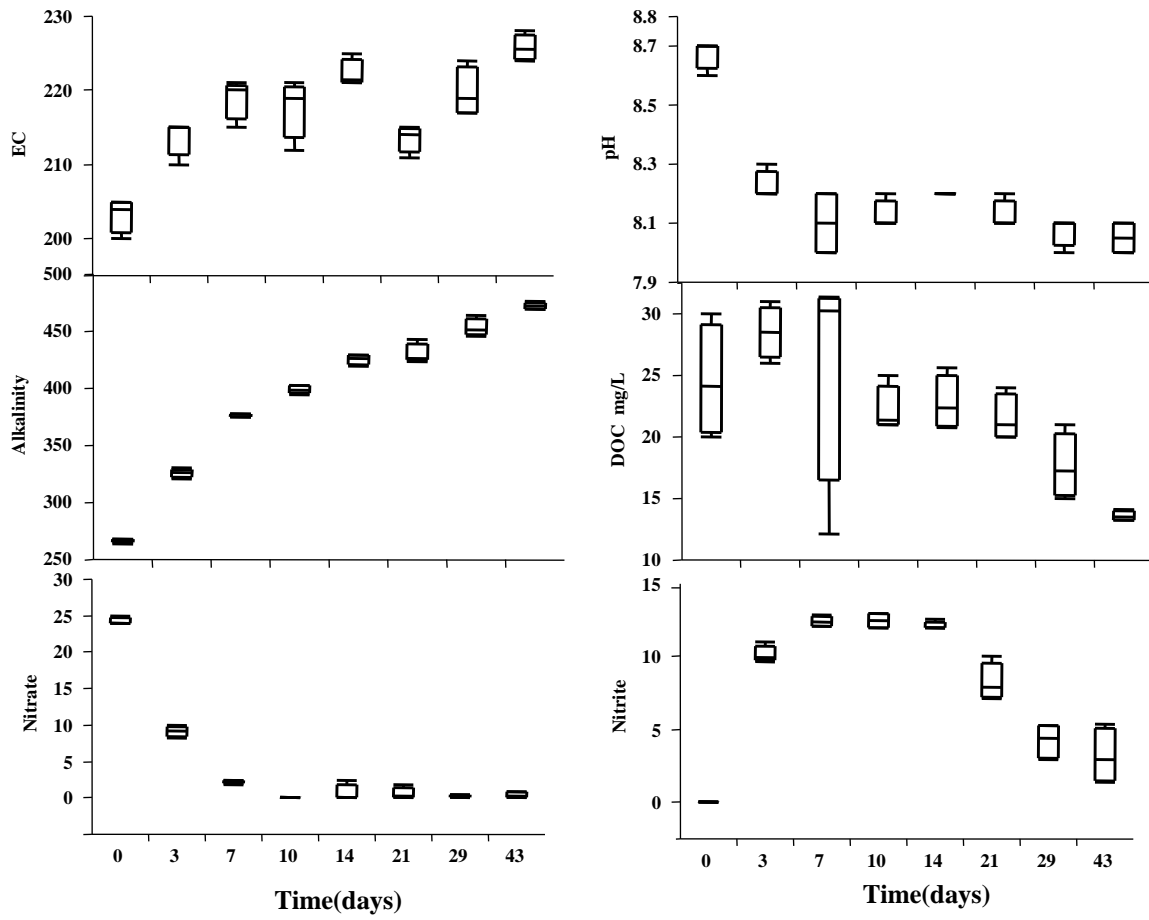


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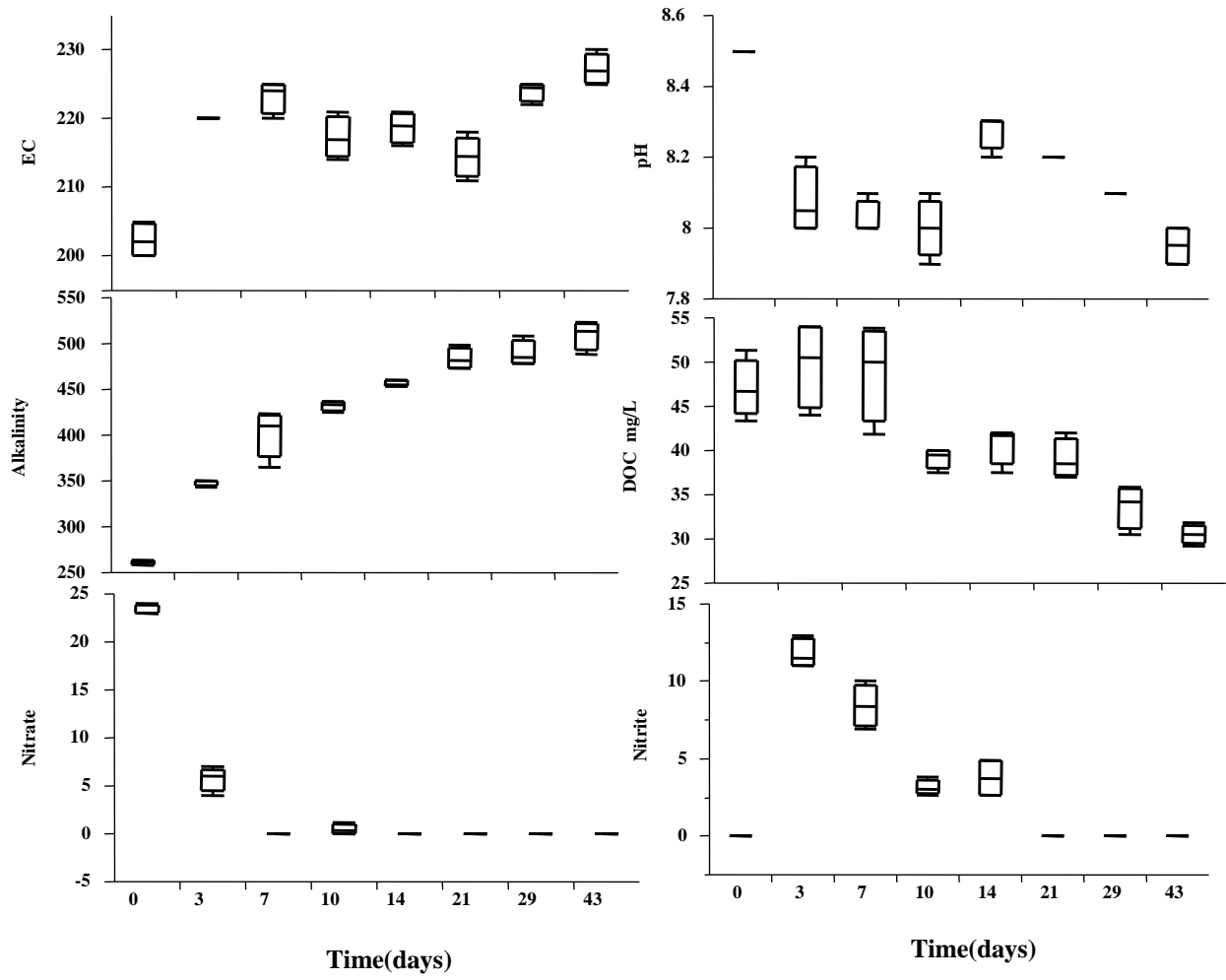


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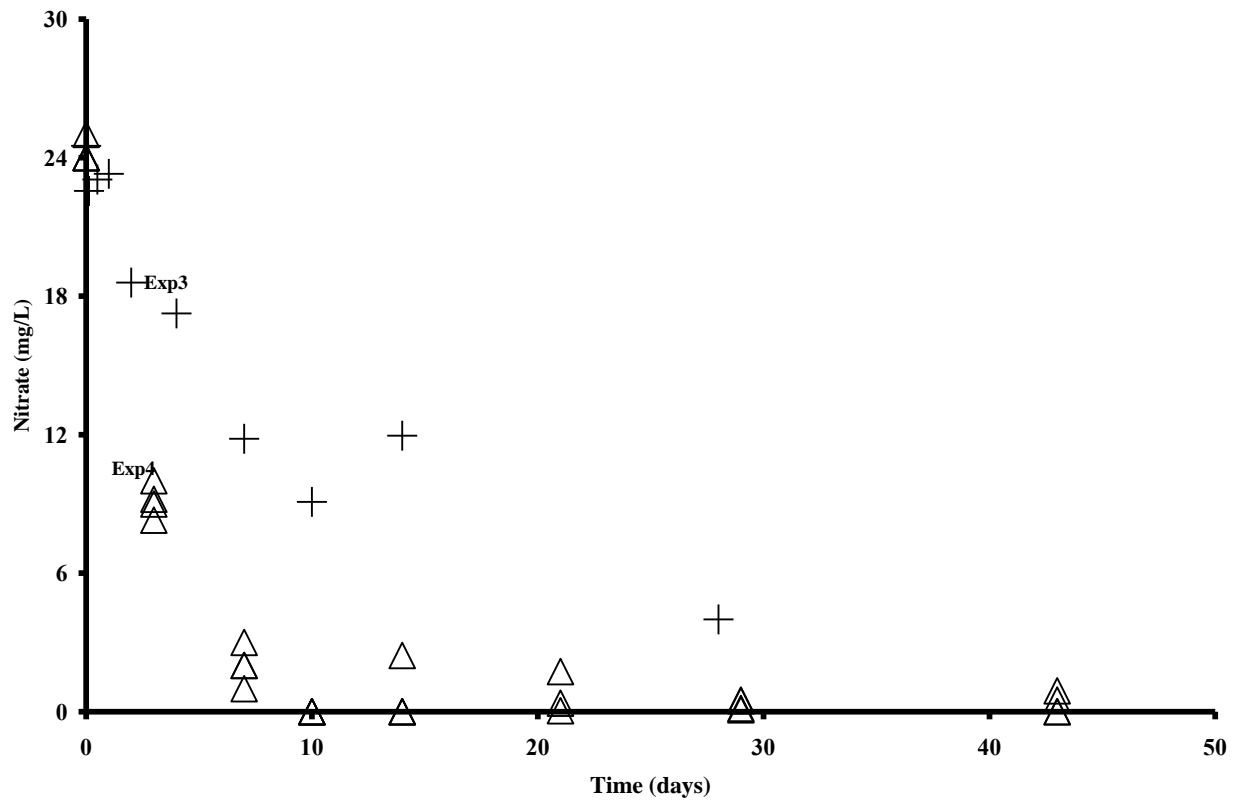


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Figure 14

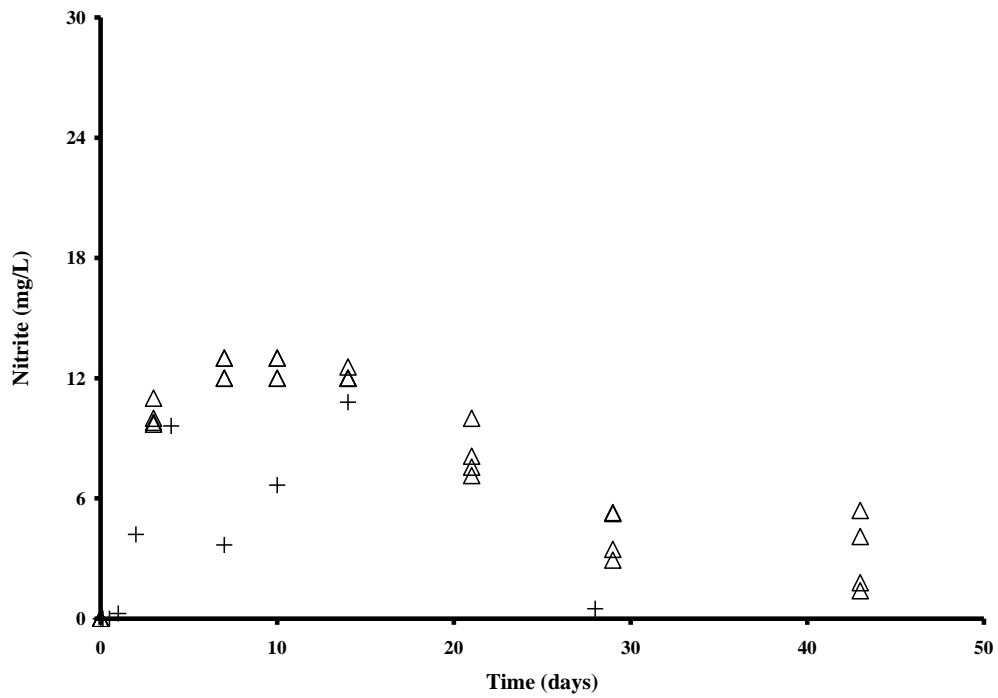


Figure 14: Nitrite-N for the duration of incubation. + represents 0.3g sawdust, 40mL groundwater, 10g soil(75-100cm layer) and 28 days incubation in experiment 3, while the Δ represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer) and 43 days incubation in experiment 4 done in triplicate.

Figure 15

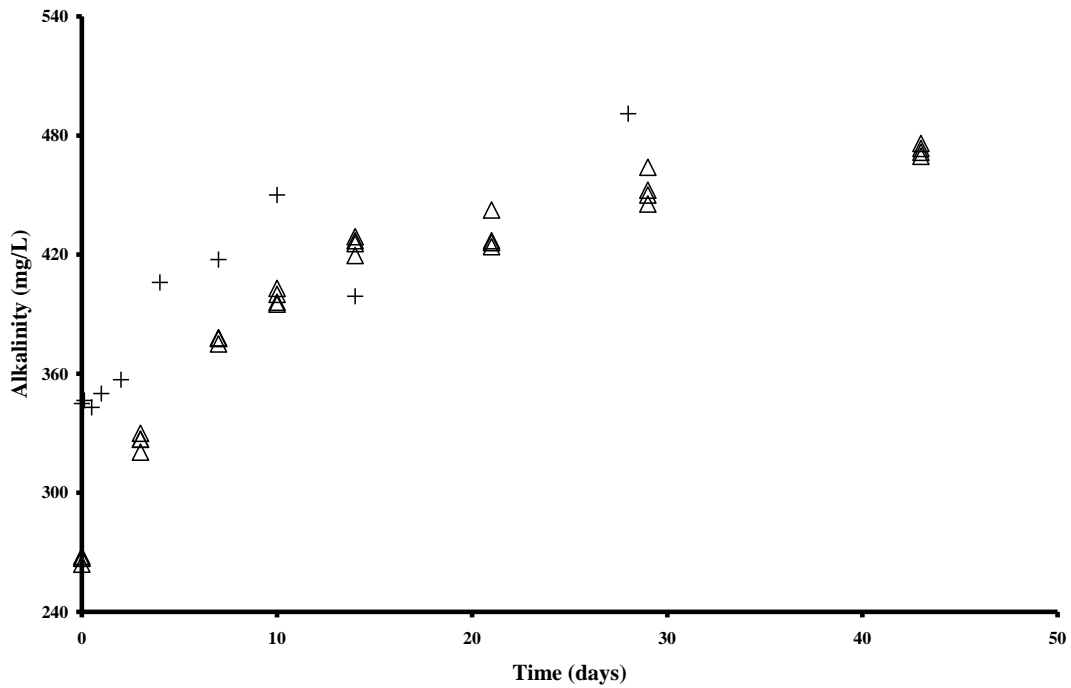


Figure 15: Alkalinity as CaCO_3 mg/L for the duration of incubation. The + sign represents 0.3g sawdust treatment containing 40mL groundwater, 10g soil (75-100cm layer) incubated for 28 days (experiment 3), the Δ represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer) incubated for 43 days and done in triplicate.