1 **PHOSPHORUS DYNAMICS NEAR BALD CYPRESS ROOTS IN A RESTORED WETLAND**

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41 **ABSTRACT**

42 Phosphorus (P) dissolution occurs commonly in wetland soils restored from agricultural land.

43 Associated with the P release are high concentrations of dissolved organic carbon (DOC) and

44 Fe^{2+}. This field study evaluated the effect of a fluctuating water table on the root dynamics of

45 bald cypress (*Taxodium distichum* L. Rich.) to determine whether root death created soil

46 reduction microsites potentially contributing to P dissolution. The study site is a restored

47 Carolina bay wetland with organic soils. Root growth and death were monitored on 16, 6-year-

48 old bald cypress using minirhizotrons. Root dynamics, water table levels, and soil porewater

49 chemistry and redox potential in the root zone were monitored for two years. Soil solution

50 samples were analyzed for Fe^{2+}, pH, DOC, and P. High rates of root growth occurred during dry

51 conditions, while root death occurred during sustained periods of saturation, particularly within

52 20 cm of the surface. Cyclic changes in concentrations of Fe^{2+}, DOC, and dissolved total P

53 (DTP) were related to water table position, but not to changes in root numbers. Following

54 sustained periods of saturated conditions, redox potential decreased to 0 mV, Fe^{2+} increased to

55 1.75 mg Fe^{2+} L^{-1}, and DOC increased to 350 mg L^{-1}; resulting in peak DTP concentrations of 750

56 µg L^{-1}, compared to 100 µg L^{-1} during dry periods. This study showed that in these high carbon

57 soils (approximately 20% organic C), rooting dynamics had minimal impact on changes in P

58 concentrations, and P dissolution was largely controlled by Fe-reduction processes occurring

59 within the C-rich soil matrix.

60

INTRODUCTION

Wetlands provide crucial ecosystem services such as wildlife habitat, groundwater recharge, and surface water quality improvement (Galatowitsch and van der Valk, 1994). To protect those services, federal and state regulations encourage wetland restoration to mitigate for the loss of existing wetlands (Dahl and Allord, 1996). However, wetlands restored from agricultural land have been observed to contribute phosphorus (P) to surface and drainage water, thus further impairing water quality (e.g., Bruland et al., 2003; Aldous et al., 2007; Ardon et al., 2010). In most of these restored wetlands, P dissolution was attributed to Fe reduction (Reddy and DeLaune, 2008), though other mechanisms have been proposed (e.g. Jackson, 1964; Greaves and Webley, 1965; Raghu and MacRae, 1966; Ponnamperuma, 1972; Turner and Gilliam, 1974a; b; Stumm and Morgan, 1981; Borggaard et al., 2005). Soil reduction microsites (approximately 25 mm in diameter) form near areas of high concentrations of labile C, such as around dead roots or in the rhizosphere where root exudates are high in concentration (Parkin, 1987). Because P dissolution in reduced soils is associated closely to Fe reduction, it is likely that P dissolution can occur at higher rates in these soil reduction microsites.

Bald cypress (*Taxodium distichum* L.Rich.) is a deciduous conifer commonly found in the coastal southeastern U.S. in Carolina bays and other low-lying areas (Elias, 1980). The species is known for extreme tolerance to flooding conditions due to its multiple metabolic and physiological adaptations (Hook, 1984). Metabolic adaptations include anaerobic respiration and increased alcohol dehydrogenase activity (Pezeshki et al., 1996) and the ability to accumulate malate and shikimate in its roots (Li et al., 2010). Physiological adaptations include the development of aerenchyma and pneumatophores, among others (Hook, 1984). Aerenchyma are porous tissues in the stem and roots that allow diffusion of oxygen into the roots and rhizosphere, and diffusion of sulfides, methane, and other toxic gases out to the atmosphere (Anderson and

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85   Pezeshki, 2000; Colmer, 2003). Pneumatophores also allow CO_2, methane, and sulfide exchange

86   with the atmosphere (Brown, 1981; Purvaja et al., 2004; Mitsch and Gosselink, 2007).

87   Most studies examining root dynamics of bald cypress have focused on container or root-

88   box rhizotron methods to study the roots (Megonigal and Day, 1992; Pezeshki et al., 1996;

89   Moorberg et al., 2013, 2015; Slusher et al., 2014). These studies allowed environmental

90   conditions to be controlled during the experiment, such as water table depth or salinity. However

91   the studies use seedlings and saplings due to constraints on the size of the trees that can be

92   studied (Böhm, 1979a), and such trees may not have developed the adaptations needed to survive

93   anaerobic conditions.

94   Root systems of older trees can be studied *in-situ* using minirhizotron tubes, which are

95   clear tubes installed in the soil at an angle into the root system (Iversen et al. 2012). The use of

96   modified cameras allows for imaging of roots at a specified depth over time (Böhm, 1979b;

97   Iversen et al., 2012). Use of minirhizotron tubes has proven useful in wetland systems which

98   experience large fluctuations of root growth and death due to shallow water tables (Baker et al.,

99   2001; Iversen et al. 2012). Root dynamics of bald cypress have not previously been examined

100 using minirhizotron tubes *in-situ*. Further, the effects of tree root dynamics on the creation of soil

101 reduction microsites and the resulting dissolution of P has not been examined in the field in

102 conjunction with root-box rhizotrons.

103   Moorberg et al. (2015) used root-box rhizotron studies to examine the effects of the

104 rhizosphere of bald cypress on P dissolution, in mineral and organic soils, simulating flooded

105 conditions of restored Carolina bay wetlands. They observed P dissolution in both the matrix and

106 rhizosphere of bald cypress in both mineral and organic soils. They also observed significant root

107 death at depths greater than 42 cm and vigorous root growth near the surface within weeks

108   following saturation. This indicated that root "redistribution" was occurring in response to

109   changes in water table depths. Areas of vigorous root growth were associated with decreases in

110   dissolved P relative to matrix concentrations following three months of saturated conditions.

111   The goals of this study were to examine the effects of root dynamics of bald cypress *in-*

112   *situ* in a wetland restored from agricultural land, and to determine if increases in Fe^{2+}, dissolved

113   organic carbon (DOC), and dissolved P occurred following root death. The hypotheses tested

114   were: i) saturated and reduced conditions would result in root death in deep soil layers and

115   concurrent root growth near the soil surface, and ii) soil depths containing dead roots would

116   exhibit increased concentrations of DOC, Fe^{2+}, and increased P dissolution.

117   **METHODS**

118   *Site Description*

119   Juniper Bay (Figure 1) is a Carolina bay in Robeson County, NC, located approximately

120   10 km south of Lumberton (34˚30'30"N 79˚01'30"E). In 1999, the North Carolina Department

121   of Transportation (NCDOT) purchased this drained Carolina bay wetland to mitigate the

122   destruction of nearby wetlands caused by highway construction (Ewing, 2003). Juniper Bay is

123   oval-shaped, oriented lengthwise along a northwest-southeast transect, and is virtually flat with

124   an area of 256 ha. Organic soils (loamy, mixed, dysic, thermic Terric Haplosaprists) occupy

125   approximately 60% of Juniper Bay, largely in its center; mineral soils (sandy, siliceous, thermic

126   Aeric Alaquods) occupy the remainder. Soil properties are shown in Table 1. The wetland was

127   drained for agriculture beginning in 1971 (Figure 1), and was fertilized annually to meet soil-test

128   recommendations. It remained in crop production until 2001, at which time restoration activities

129   commenced (Ewing, 2003). Extensive background characterization of soils and monitoring of

130   hydrology in the bay was performed for five years prior to restoration. Preliminary restoration

131 efforts started in June 2003, and ditch filling began in late 2005. During and after restoration the

132 water quality has been monitored at the single, surface-water outflow structure on the southern

133 edge of the bay, and in groundwater samples throughout the site. Concentrations of P in the

134 surface water outflow have shown that P was lost from the bay following restoration of wetland

135 hydrology (Moorberg, 2014).

136 *Experimental Design*

137   The plot locations were determined by placing an equilateral triangle grid over the soils

138 map of the bay, and then selecting eight locations that were distributed across the organic and

139 mineral soil units (Figure 1). Four plots were in organic soils and four in mineral soil.

140   Bald cypress was chosen as the study species because it is one of the more common trees

141 planted at Juniper Bay during the restoration (N.C. Department of Environment, Health, and

142 Natural Resources (DEHNR), 2010), and has been previously studied in rhizotron and container

143 studies using Juniper Bay soils (Moorberg et al., 2013, 2015; Slusher et al., 2014). At each plot,

144 an initial tree survey was performed within 30 m of the existing groundwater monitoring wells to

145 identify bald cypress trees that were in good health and were 3 m in height or taller. Of the

146 eligible trees, two per plot were randomly selected for instrumentation.

147 *Minirhizotron Construction and Installation*

148   A minirhizotron system was installed for monitoring root growth and death throughout

149 the study (Figure 2). The minirhizotron tubes were 1.5 m long, acrylic 5.08 cm ID x 5.72 cm OD

150 (Piedmont Plastics, Morrisville, NC, USA). A hole was drilled at the top of each tube to engage

151 the locking mechanism of the minirhizotron camera indexing handle system. A 5.08 cm diameter

152 mechanical test plug (Oatey Supply Chain Services, Cleveland, Ohio, USA) coated with vacuum

153 grease was used to seal the bottom of the rhizotron tube. The tubes were installed at each

154   instrumented tree by boring an auger hole 60 cm from the base of the tree trunk with a 5.08 cm

155   diameter soil auger held at a 45˚ angle. A jig was used to hold the auger as close to 45˚ as

156   possible. Each rhizotron tube was then inserted into the auger hole. Exposed portions of each

157   tube were covered with adhesive aluminum flashing foil to limit light entering the tube, and to

158   offer some insulation. Each tube was secured with zip ties to wooden stakes.

159   *Root Analysis and Tree Measurements*

160   Roots were photographed monthly for a 2-year period on the days soil porewater was

161   collected. Images were obtained with a BTC-2 Camera System (Figure 2), BTC I-CAP software,

162   and the Indexing Handle System (Bartz Technology Corporation, Santa Barbara, CA, USA).

163   This system captures images of roots within "windows" 13.5 mm vertical by 18 mm horizontal

164   in size. Within the BTC I-CAP software, each root image is tagged with a minirhizotron tube

165   number, a session number, and a window number for future analysis.

166   Each image was analyzed for root length, diameter, color, growth, and death using

167   RootFly 2.0.2, a free, open-source software application designed for minirhizotron image

168   analysis. While RootFly does offer an automated image analysis algorithm for tracing new roots,

169   that feature was determined to have limited utility for this application. Thus, all roots were

170   identified and traced manually. The root data was summarized for depth intervals of 0-20 cm,

171   20-40 cm, and 40-60 cm for all tubes over all sessions. Root depth was determined using the

172   following equation:

$$D_r = W_n * 1.35 * \tan(\alpha) \qquad\qquad \text{Eq. 1}$$

173   where D_r is the vertical root depth in cm, W_n is the window number, 1.35 is the window height in

174   cm, and α is the angle at which the minirhizotron tube is installed. In this study, a 45° angle was

175   used, which simplifies equation 1 to:

$$D_r = W_n * 1.35 \hspace{4cm} \text{Eq. 2}$$

176 Summary data included total root number, total root length, and average width for each depth.

177   The diameter at breast height (DBH) and tree height were determined for each

178 instrumented tree on January 23, 2012, and again on June 17, 2013 using calipers, and measuring

179 tape and a clinometer, respectively.

180 *Soil Solution Sampling and Redox Measurements*

181   Soil solution was collected using Rhizon soil porewater samplers (standard 2.5 mm

182 rhizon sampler, 5 cm length, Rhizosphere Research Products, Wageningen, The Netherlands).

183 Before installation, Rhizon samplers were stored for 24 hours in deionized water with the

184 hydrophilic tips submerged to saturate them for use. The tubing of each sampler was extended

185 using approximately 40 cm of polytetrafluoroethylene (PTFE) tubing (0.8mm I.D.) to keep the

186 Luer lock of each sampler above the soil surface and/or ponded water. Rhizon sampler porous

187 tips are made of hydrophilic plastic (Shotbolt, 2009) which do not absorb P from solution as do

188 porous ceramic samplers (Zimmermann et al., 1978; Nagpal, 1982; Bottcher et al., 1984). As

189 described by Shotbolt (2009), the sampling tips have a mean pore size of 0.15 μm making

190 additional sample filtration unnecessary. Further, the Rhizon samplers do not change sample

191 redox conditions under normal sampling environments, and provide many other improvements

192 over porous cups for pore water sampling.

193   Platinum-tipped redox electrodes similar to that of Wafer et al. (2004) were used to

194 monitor redox potential. Measurements were made using a calomel reference electrode and a

195 voltmeter. Voltage readings were corrected relative to a standard hydrogen electrode by adding

196 250 mv to all field readings, as described by Vepraskas and Faulkner (2001). These readings

197 were not corrected for temperature. Again, based on redox corrections described by Vepraskas

198   and Faulkner, temperature differences in the range of 10 to 25°C would have resulted in

199   negligible differences in redox potentials of approximately 10 mV.

200   One soil solution sampler and three redox electrodes were installed at depths of 15 and 30

201   cm, at a distance of 30 cm from the edge of each tree trunk as depicted in Figure 2. A

202   polycarbonate plate (LEXAN, SABIC, Riyadh, Saudi Arabia) was first installed vertically in the

203   soil to hold the Rhizon samplers and redox electrodes at the desired depths. Each plate was

204   driven to the proper depth with a rubber mallet. Care was taken to limit root injury during the

205   installation of the plates, samplers, or electrodes. After the plate was inserted, an access hole was

206   excavated on one side of the plate to allow insertion of samplers and electrodes through holes

207   that were pre-drilled into the plate at the proper depths. The plates also allowed soil on one side

208   to remain undisturbed, while the access hole was refilled. After installation, the access hole was

209   backfilled with soil. The tubing and wires from the samplers and redox electrodes were secured

210   to a support constructed of polyvinyl chloride (PVC) pipe to ensure that wire and tube tips would

211   remain out of ponded water. Rhizon samplers were replaced every six months as recommended

212   by the manufacturer.

213   *Sampling and Analyses*

214   Soil pore-water sampling was performed monthly beginning in May 2011 and continued

215   for two years. Two amber-colored serum bottles (100 ml and 30 ml) were used to collect soil

216   pore water samples from each sampler. Prior to sampling, each 100 mL bottle was acid-washed

217   and dried, acidified with 0.250 ml of 12 M H_2SO_4, capped with a rubber septa and aluminum

218   cap, and evacuated to -78.5 kPa or greater pressure using an electric vacuum pump. The 100 ml

219   bottle was used for collecting samples for the analysis of dissolved Fe^{2+}, dissolved reactive P

220　(DRP), and dissolved total P (DTP). The second bottle was left un-acidified and used to collect

221　samples to measure dissolved organic C (DOC) and pH.

222　　　Water samples were collected through a 25-gauge, 3.8 cm long, Luer-lock needle that

223　was attached to each Rhizon sampler. The sampling tube was first purged by inserting the needle

224　into an evacuated serum "purge" bottle to collect one Rhizon sampler volume (0.187 ml) (Soil

225　Moisture Equipment Corp., 2008) or more of water. After purging, the sampler needle was

226　inserted into the septa of the 30 ml serum bottle to collect approximately 15 ml of solution over

227　the course of four to six hours. Following this, the needle was inserted into the 100 ml bottle to

228　collect approximately 30 ml of solution overnight and collected first thing the next morning. The

229　unacidified samples were frozen upon the return from the field, and remained frozen until ready

230　for analysis. The acidified serum bottles were stored at room temperature in the dark until they

231　were analyzed for Fe^{2+}.

232　　　The concentration of Fe^{2+} in solution was determined using the phenanthroline method

233　(Joint Task Group: 20th Edition, 2005). The samples were reacted with the phenanthroline

234　reagent within 24 h of collection, and analyzed using a Shimadzu UV-2101PC

235　spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) within 48 h of

236　sampling. A sample aliquot without phenanthroline reagent was used as a blank to correct the

237　absorbance reading for the impact of dissolved organic matter. Standard curves were produced

238　using ammonium iron (II) sulfate hexahydrate (99.997%). The remaining acidified samples were

239　then transferred to 20 mL scintillation vials for storage for future P determinations.

240　　　Subsamples for P determinations were submitted to the Environmental and Agricultural

241　Testing Service at NC State University. Dissolved reactive phosphorus was analyzed using a

242　multi-channel Quick Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) using the method

243   described by Prokopy and Wendt (1994). Dissolved total P was analyzed using an Inductively

244   Coupled Plasma-Atomic Emission Spectrometer (Optima 2000, Perkin-Elmer, Waltham, MA,

245   USA).

246   The frozen samples were thawed and allowed to return to room temperature. Solution pH

247   was measured with a pH electrode. Because pH was not determined immediately in the field, soil

248   solution pH from the 30 cm depth samples were compared to samples from a concurrent study

249   that were collected from a well at the same depth that were analyzed for pH immediately. There

250   was no significant difference, therefore the authors conclude that freezing samples prior to

251   analyzing pH did not compromise sample pH. Following pH measurements, each sample was

252   immediately analyzed for DOC using a Shimadzu TOC-5050 total organic carbon analyzer

253   (Shimadzu Scientific Instruments, Columbia, MD, USA). Flocculation of DOC due to freezing

254   has been observed by Giesy and Briese (1978), but was not observed in this study. The standard

255   definition of dissolved organic carbon is organic C that passes through a 0.45 µm filter. Because

256   the Rhizon samplers used in this study have a smaller pore size (0.15 µm), in this paper we

257   operationally define DOC as dissolved organic C that passes through a 0.15 µm filter. The

258   smaller pore size in Rhizon samplers is inherent in the sampler design, but allows for porewater

259   collection under unsaturated conditions (Seeberg-Elverfeldt et al., 2005), a tradeoff the authors

260   deemed appropriate.

261   *Statistical Analysis*

262   The experimental design was a split-split plot design with the fixed effects including two

263   soil treatments (mineral and organic), two soil depths (15 cm and 30 cm) for soil solution

264   measurements, and the sampling date. Three depths were used for root data (0-20 cm, 20-40 cm,

265   and 40-60 cm). The two soil treatments were split among eight plots with four plots located on

266   each soil type. Each plot was replicated with two instrumented trees per plot location. The data

267   were analyzed in SAS 9.3 (SAS Institute Inc., 2011, Cary, NC, USA) using the PROC MIXED

268   procedure. Error bars shown in each figure depict the standard error of the mean. Multiple

269   comparisons were adjusted using the Tukey method in SAS. Natural log transformations were

270   used for DTP, DRP, DOP, and Fe^{2+}, and square root transformations were used for root counts

271   and root length sum data to conform to the normality assumptions of PROC MIXED in SAS.

272   **RESULTS**

273   *Rainfall and Water Table Depths*

274   The daily rainfall and the water table depths from the manual wells recorded at the time

275   of sampling are shown in Figure 3. Rainfall observed at Juniper Bay was below normal for both

276   2011 (956 mm) and 2012 (1020 mm) according to the USDA NRCS WETS table for Robeson

277   County (USDA-NRCS, 2013), which reports a normal rainfall range of 1085-1290 mm. As a

278   result, the average water table depth only went above the 15 cm depth (depth of the shallowest

279   soil porewater sampler) of the mineral soil once during the two-year study. Shallow water tables

280   did occur in the organic soils. This was likely due to the lower position of the organic soils on the

281   relatively flat Carolina bay landscape. Because one of the primary objectives of this study was to

282   observe changes in soil solution chemistry under saturated conditions in the field, the focus of

283   this paper going further will be on the organic soil results.

284   Saturated conditions occurred at the 30 cm depth from the beginning of the study in May

285   through June 2011, February 2012 through September 2012, and again from January through

286   May 2013. Saturated conditions occurred at the 15 cm depth for shorter durations than the 30 cm

287   depth. At 15 cm the organic soils were saturated at the first sampling in May 2011, then from

288   March 2012 through July 2012, then again from January 2013 through the end of the study in

289   May 2013.

290   *Redox Potential and pH*

291   The redox potentials at both the 15 and 30 cm depths over time are shown in Figure 4. On

292   average, redox potentials were significantly lower at 30 cm (158 mV, se ±34 mV) than 15 cm

293   (378 mV, se ±34 mV) (p<0.0001) across all sampling events and both soil types. The lowest

294   redox potentials (approximately 0 mV for both depths) occurred during late spring and early

295   summer when the water table was within 30 cm of the surface and temperatures were increasing.

296   At a depth of 30 cm, redox potentials were low enough for reduced Fe^{2+} to occur, <300 mV at

297   pH 4 (Vepraskas and Faulkner, 2001), from May to June 2011, and from January 2012 through

298   the conclusion of the study. At the 15 cm depth, Fe^{2+} would be expected in solution from May to

299   June 2011, March through November 2012, and from April through May 2013. The 15 cm depth

300   reached a maximum redox potential of approximately +700 mV during the fall months of 2011 –

301   the driest period of the study. The 30 cm depth reached +500 mV for the same period.

302   *Tree and Root Measurements*

303   The instrumented trees averaged 7.4 cm (se ± 0.2 cm,) DBH in January 2012, and

304   increased to 10.9 cm (se ± 0.2 cm) DBH by July of 2013. Tree heights were 5.5 m (se ± 0.4 m) in

305   January 2012, and increased to 5.7 m (se ± 0.4 m) by July 2013. Root counts from the

306   minirhizotron images are shown in Figure 5 for the 0-20, 20-40, and 40-60 cm depth intervals

307   over time with a square root-transformation. Root counts were highest at the surface and

308   decreased with depth for any specific time. The net changes over time are shown in Figure 6.

309   The largest increase in root growth occurred during the dry period in the summer months of

310   2011, followed by another smaller increase in root counts in the summer of 2012. Root death

311   occurred at all depths from August 2012 to May 2013, but was most pronounced in the upper 20

312   cm.

Dissolved Organic Carbon

314   Dissolved organic carbon (Figure 4) declined during the dry period of 2011 to minimums

315   of 150 mg DOC L^{-1} in the 15 cm depth, and 250 mg DOC L^{-1} in the 30 cm depth. Following a

316   rise in the water table and decreases in Eh at the two sampled depths in February 2012, DOC

317   rose to peaks of approximately 350 mg DOC L^{-1} by June 2012. There was no significant

318   difference in DOC between depths.

Ferrous Iron

320   Reduced Fe concentrations (shown with a natural log-transformation in Figure 4) were

321   highest after sustained periods of saturated conditions and low redox potentials. Concentrations

322   in back-transformed values ranged from approximately 0 mg in the late summer of 2011 to 1.75

323   mg Fe^{2+} L^{-1}. In August and September 2012 the water table was between the 15 and 30 cm

324   depths creating oxidizing conditions in the 15 cm depth (Figure 3) and resulting in decreases in

325   Fe^{2+} at that depth (Figure 4). In the 30 cm depth, Fe^{2+} remained high with continued saturated

326   and reduced conditions. Concentrations declined slightly in November 2012 at both depths, then

327   increased again in May when water tables rose again.

Phosphorus

329   Dissolved total P concentrations (Figure 4) increased with increases in Fe^{2+} concentration

330   and decreases in redox potential. In addition, higher DTP concentrations (p=0.0008) were found

331   at the 30 cm depth than at the 15 cm depth across all sampling dates. This was likely due to the

332   30 cm depth having a lower redox potential (Figure 4). The highest DTP concentrations occurred

333   following sustained reduced conditions, particularly in May-July 2011, and again from August

14

334   2012 through February, 2013. Concentrations (in back-transformed values) of DTP ranged from

335   100 to 750 µg L^{-1}. Concentrations of DRP largely followed the same trends as DTP, but

336   differences were less pronounced (Supplemental Figure S1).

337   **DISCUSSION**

338   We hypothesized originally that dead roots would form microsites where Fe would be

339   reduced and DTP released. As a result, we expected to see concentrations of Fe^{2+} and DTP

340   increasing after roots had died. This was not observed, in part, because root growth and death

341   were not controlled by saturation and water table levels in the soil studied. Root numbers

342   increased from May 2011 through July 2012 at all depths, particularly in the top 20 cm, and

343   decreased thereafter through the conclusion of the study in May 2013. Drier than normal

344   conditions existed at Juniper Bay during this study, particularly during the late summer and fall

345   of 2011 when the water table dropped below 80 cm in the organic soils. Under dry conditions it

346   is common for trees to drop some leaves and reallocate resources to the production of new roots

347   (McDowell et al., 2008). However, root numbers continued to rise from February to May in

348   2012, even after the water table rose above a depth of 40 cm. Megonigal and Day (1992)

349   observed that bald cypress that experienced alternating flooded and dry soil conditions exhibited

350   increased root production relative to continuously flooded sites, because intermittent flooding

351   allowed the trees adequate moisture during times when the soil was not waterlogged.

352   Changes in root numbers were not related to redox potential. Increases in root numbers

353   from May 2011 through July 2012 occurred during a period when the redox potential increased

354   from approximately 0 to 700 mv (15 cm depth) and declined back to approximately 0 mv. This

355   indicated the root increases in the upper 20 cm occurred during periods of aerobic as well as

356   anaerobic, Fe-reduced conditions. The bald cypress roots of the trees studied were adapted to

357   grow under anaerobic conditions. Further, had this study been conducted during a wetter period,

358   similar results in root growth would be expected, due to root numbers having no relation to redox

359   potential.

360   In root-box rhizotron studies, several researchers have observed root growth in the soil

361   surface concurrent with root death in deeper, more reduced soil layers (Schat, 1984; Moorberg et

362   al., 2013, 2015; Slusher et al., 2014). However, that pattern of root redistribution was not

363   observed in this minirhizotron-tube study. This could be due to the age and pre-conditioning of

364   the trees studied, which allowed the roots to develop adaptations (e.g., arenchyma) that

365   conditioned them to survive in anaerobic environments. In root-box rhizotron studies and other

366   container studies, tree size are limited to seedlings and saplings which may be experiencing

367   saturated soil conditions for the first time, and must develop adaptations or shift root distribution

368   to adjust to anaerobic conditions. The 6-year-old trees at Juniper Bay had already experienced

369   six seasonal water table fluctuations, while the saplings used in previous root-box rhizotron

370   studies (Schat, 1984; Moorberg et al., 2013, 2015; Slusher et al., 2014) were experiencing

371   saturation for the first time. Thus, pre-conditioning tree saplings prior to use in container studies

372   simulating wetland hydrology may improve the relevancy of such experiments to field

373   conditions.

374   DOC fluctuations were not clearly related to root growth. We expected to see DOC

375   concentrations increase following periods of root death but this was not observed. The largest

376   increases in DOC occurred from January through September 2012 when root numbers at all

377   depths were either increasing or remaining constant. The source for the DOC may have been

378   decomposing organic tissues in the matrix of these organic soil materials.

379   Concentrations of Fe^{2+} were related to water table levels, and reached peak values

380   approximately 1 to 2 months after each depth was saturated. Once that depth became oxidized

381   following a drop in the water table, concentrations of Fe^{2+} immediately declined. Peak

382   concentrations in Fe^{2+} occurred when the soil was below a redox potential of 300 mV (Figure 4),

383   the redox potential at which Fe would be expected to be reduced for a soil pH of 4, according to

384   the assumptions listed by Vepraskas and Faulkner (2001).

385   Concentrations of Fe^{2+} varied in a pattern similar to changes in DOC with the highest

386   concentrations for both occurring within the May to September period in 2011 and 2012. This

387   pattern showed no apparent relationship to changes in root growth, again indicating that root

388   death was not supplying organic C used by microbes that utilized Fe^{3+} compounds as electron

389   acceptors. The organic C that was oxidized when Fe was being reduced apparently came for the

390   matrix of the organic soils.

391   Also during the 2012 drawdown, DTP concentrations dropped from peak concentrations

392   (600-700 µg L^{-1}) reached following three to four months of prolonged saturated, reduced

393   conditions at the 15 cm depth, while DTP concentrations remained high in the still saturated and

394   reduced 30 cm depth. Because the DOC concentration did not depict any sudden changes during

395   this period, precipitation of Fe^{3+} is the likely cause of the decreases in DTP concentration, thus

396   indicating the reduction and oxidation of Fe is controlling P concentrations in this system.

397   Increases in DTP concentrations at both depths occurred following soil saturation, with peak

398   concentrations occurring 3 to 4 months after the onset of saturation, which were within the time

399   frame noted for peaks in Fe^{2+}. Patterns in DRP concentrations through time mirrored those of

400   DTP, though at lower concentrations (Supplemental Figure 1).

401   These results for Fe, DOC, and DTP matched those of Moorberg et al. (2013, 2015), who

402   found that P dissolution, as well as the precipitation of dissolved P, was controlled primarily by

403   Fe reduction and oxidation. The presence of living and/or dead roots did not cause additional

404   dissolution of P above concentrations found in the matrix for the soil material having an organic

405   C of 19.5% (based on C measurements by Abit, (2009). Further, P concentrations under

406   sustained saturated and reduced conditions could be limited by the presence of living roots that

407   contain aerenchyma. From those results, it can be inferred that the dissolution of P in this field

408   study is the product of reduction processes occurring in the matrix, and that labile C was not

409   limiting to soil reduction in the Histosol studied. Concentrations of DOC in this study exhibited

410   minimal changes through the dry and wet seasons, while both DTP and Fe^{2+} showed large

411   variations in concentrations with changes through the seasons. Further, P concentrations

412   increased with increases in Fe^{2+} as the soil became more reduced. Upon the water table dropping

413   below the 15 cm depth in August of 2012, and the resulting oxidizing conditions, Fe began to

414   precipitate, and DTP concentrations immediately declined. Therefore, P dissolution is likely Fe-

415   controlled in this restored, Carolina bay Histosol.

416   In addition, despite this study being conducted during an abnormally dry period, we

417   expect the results to be representative of what might occur under normal rainfall due to the

418   correspondence of these observations to those of Moorberg et al. (2013, 2015), which were

419   conducted in controlled, saturated conditions in a greenhouse setting.

420   **CONCLUSIONS**

421   The goals of this study were to examine the growth and death of bald cypress roots *in-situ*

422   in a wetland restored from agricultural land, and to determine concentrations of Fe^{2+}, DOC, and

423   DTP increased after roots died. Root growth and death had no apparent effect on concentrations

424   of Fe^{2+}, DOC, or DTP. This indicated that while dead roots might be microsites for Fe reduction

425   in some soils, they had little effect in reducing processes here. This is most likely because the

426   soil matrix of the organic soil studied contained adequate labile C in its matrix to enable Fe

427   reducing reactions. Dissolution of P did occur during extended periods of saturation, and was

428   likely controlled by Fe reduction. Previous and concurrent root-box rhizotron studies showed

429   that contributions of labile C from the rhizospheres of bald cypress growing in soils with high

430   organic matter content, such as the soils used in this study, did not cause additional soil P

431   dissolution, and that is assumed to be the case for this field study as well. However, the

432   contribution of root dynamics on P dissolution in soils with low amounts of organic matter and

433   labile C is still unknown and needs further research.

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557 **TABLES**

558 **Table 1. Summary of soil chemical and physical properties for the mineral and organic**
559 **soils from Juniper Bay.†**

Soil Properties	Unit	Mineral Soil	Organic Soil
Organic C	% w/w	3.5	19.5
‡Total P	mg cm^{-3}	0.21	0.13
Mehlich III P	mg cm^{-3}	0.08	0.12
pH		4.9	4.2
Surface Texture		Loamy Sand	Sapric material
Bulk Density	g cm^{-3}	1.45	0.62
§Porosity		0.48	0.63
§Saturated Conductivity	m d^{-1}	3.00	1.17

560 †Table originally published in Moorberg et al. (2015) and provided here
561 with permission.
562 ‡Values from Moorberg et al. (2014 chap. Phosphorus Fluxes in a
563 Restored Carolina Bay Wetland Following Eight Years of Restoration)
564 §Values from Abit (2009)

591 **LIST OF SUPPLEMENTAL FIGURES**

592 **Supplemental Figure 1. Concentration (natural log-transformed) of DRP by depth over**
593 **time. The y-axis shows natural log-transformed concentrations of DRP concentration, with**
594 **time on the x-axis. The concentration of DRP increased slightly during periods of high Fe^{2+}**
595 **concentrations. The error bars depict standard error. The observed concentrations ranged**
596 **in back-transformed values of approximately 75 μg L^{-1} to 500 μg L^{-1}.**

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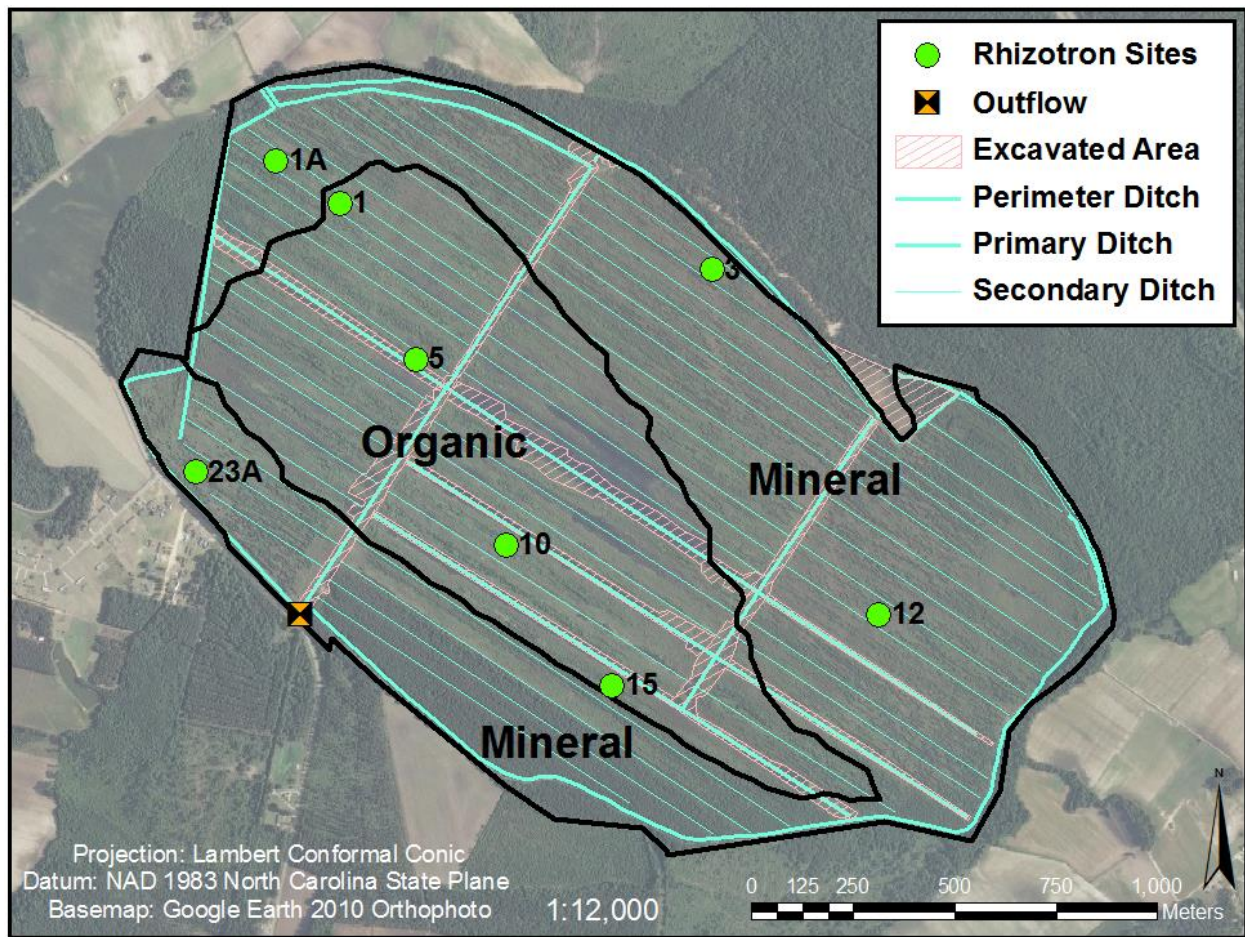
Figure 1. Map of Juniper Bay and Study Sites. Eight study sites were split between the two soil types, mineral and organic, at Juniper Bay. Two trees were instrumented at each of the eight study sites, with four sites located on mineral soils, and four on organic soils.

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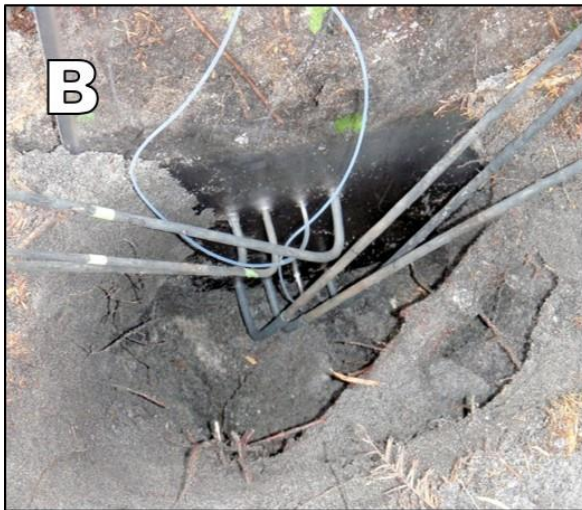
Figure 2. Minirhizotron tube, soil sampler, and groundwater well instrumentation. As shown in picture A, each studied tree was instrumented with a (left to right) groundwater monitoring [manual] well, a rhizon soil porewater sampler and redox electrode station, and a minirhizotron tube.

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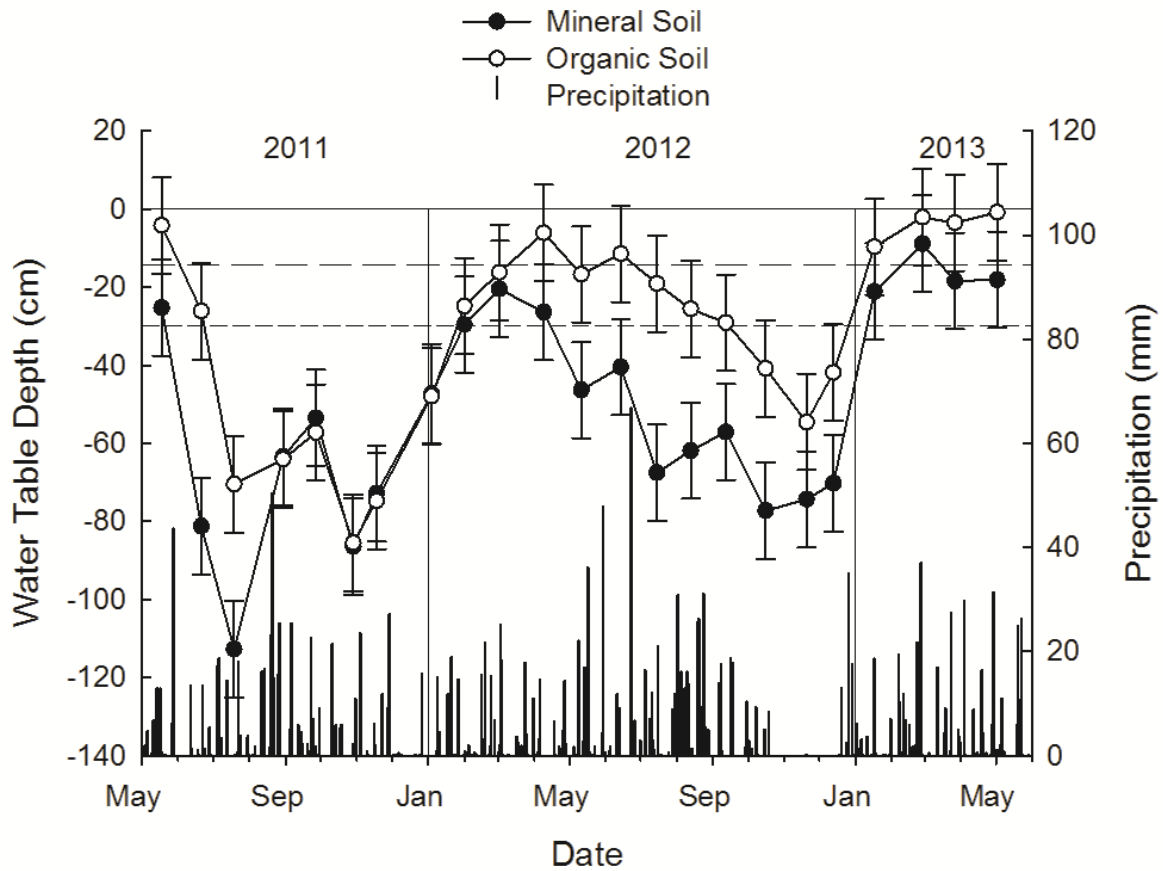
Figure 3. Average water table depths for the mineral and organic soils and daily rainfall data for Juniper Bay. The error bars depict standard error. Water table depths are shown for the duration of the field study. The solid black line at depth 0 depicts the soil surface, while the long-dashed line and the short-dashed line depict the location of the 15 cm and 30 cm depth samplers, respectively.

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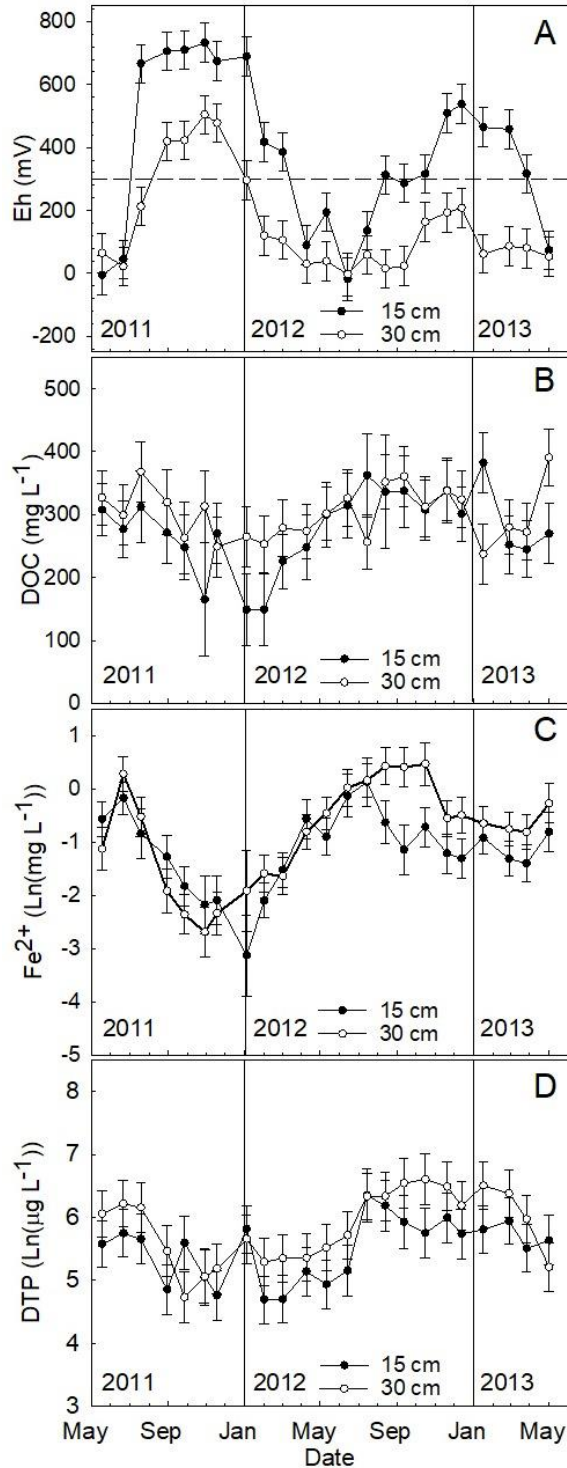
Figure 4. Redox potential (A), concentration of DOC (B), concentration (natural log-transformed) of Fe²⁺ (C), and concentration of (natural log-transformed) DTP (D) at 15 and 30 cm in depth over time for the organic soil. The error bars in each panel depict standard error.
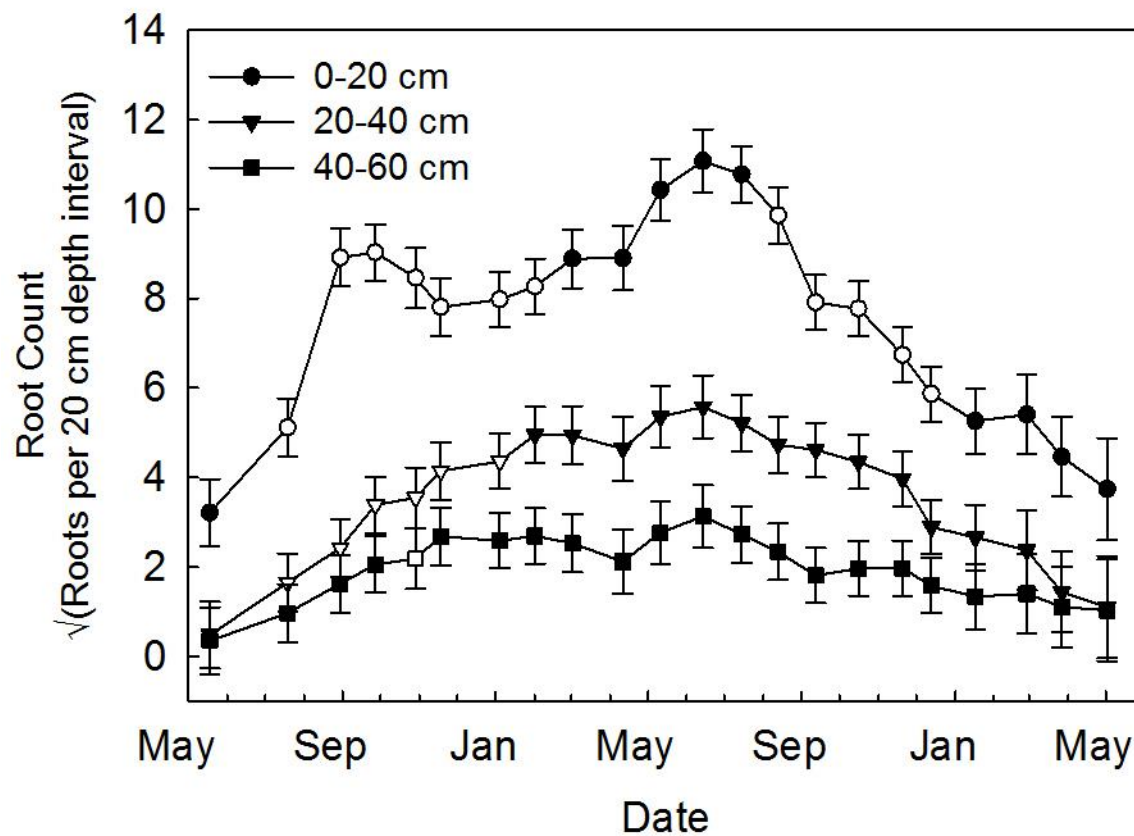
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Figure 5. Root counts (square root-transformed) from minirhizotron images for 0-20, 20-40, and 40-60 cm depths of the organic soil. The y-axis is the square root of the number of roots observed for each of the three depths for a given sampling event. Open symbols indicate that the water table is deeper than the depth interval. Error bars depict the standard error.

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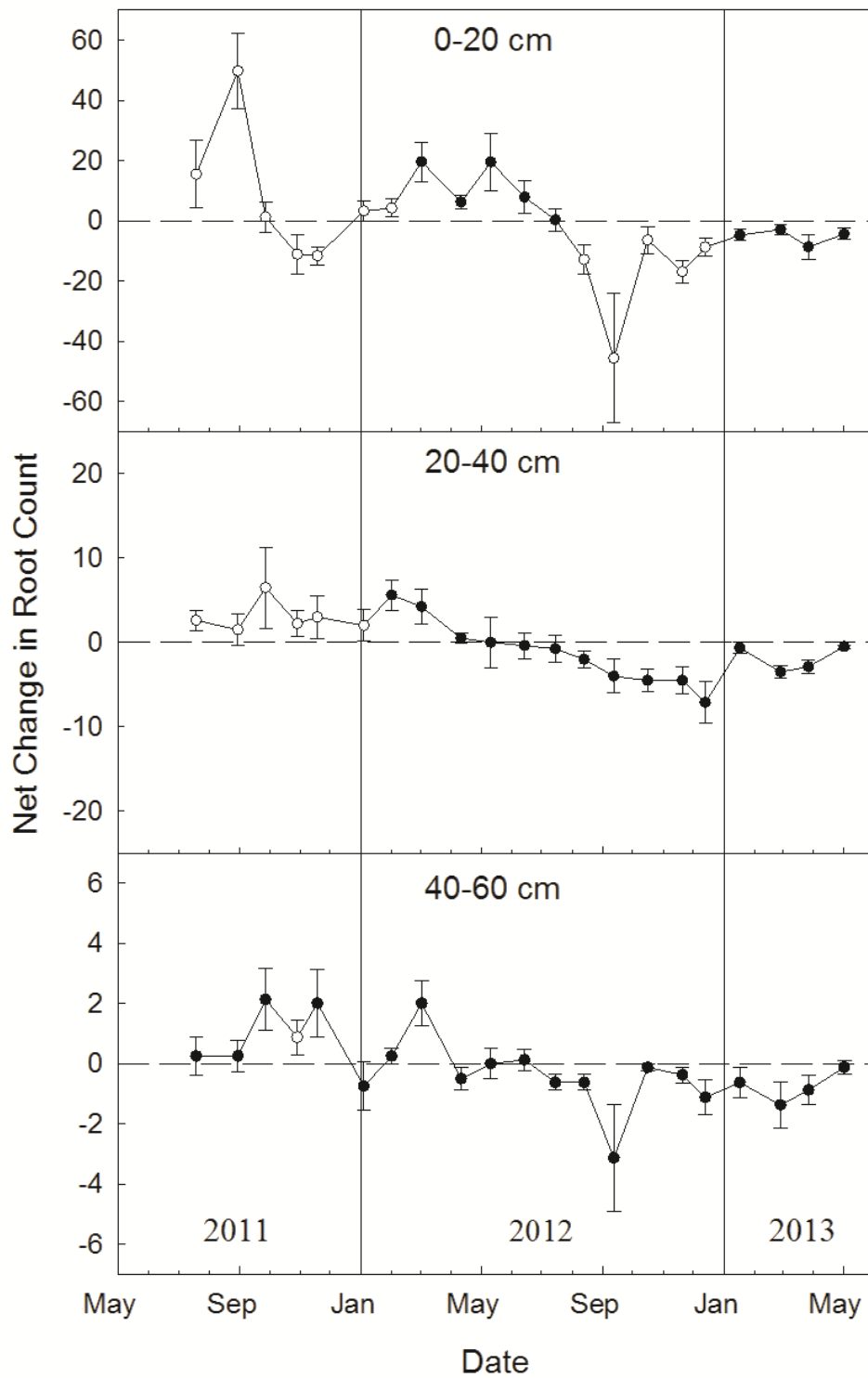
Figure 6. Change in root count by depth over time of the organic soil. The values on the y-axes depict the change in overall root count for each depth from the count of the previous month. Positive numbers depict new root growth, while negative values show root death. The scale of the y-axis is different for each depth. The error bars depict standard error.

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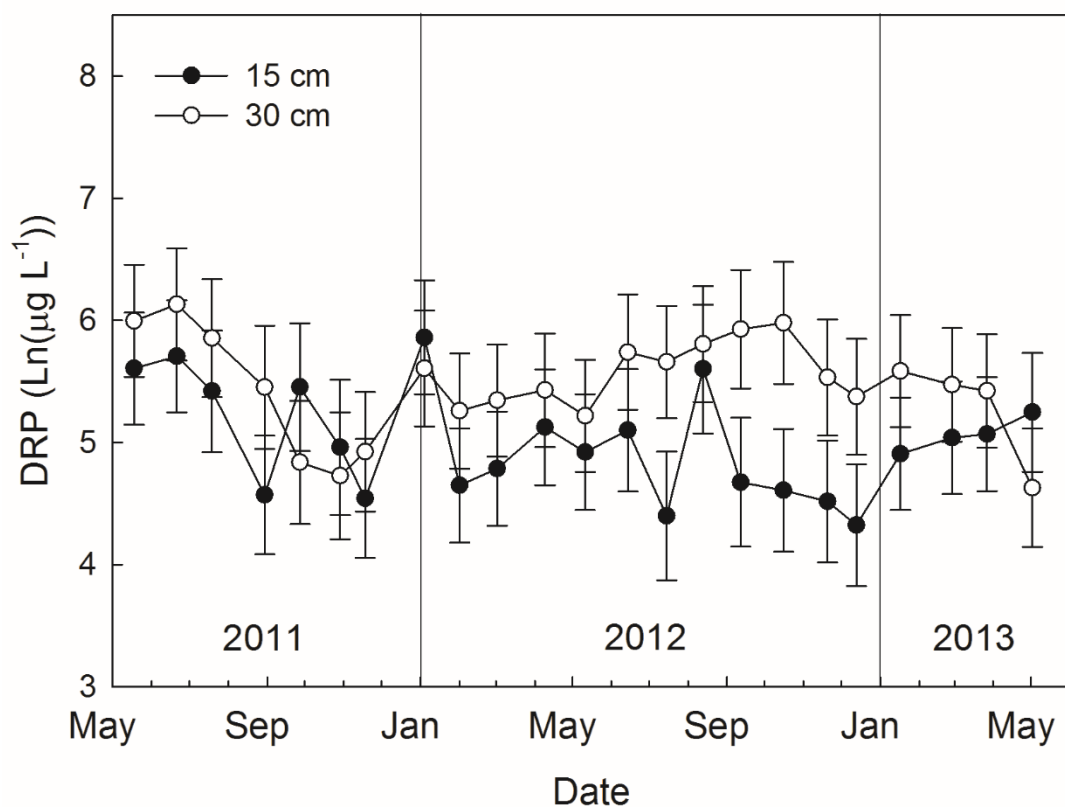
Supplemental Figure 1. Concentration (natural log-transformed) of DRP by depth over time. The y-axis shows natural log-transformed concentrations of DRP concentration, with time on the x-axis. The concentration of DRP increased slightly during periods of high Fe^{2+} concentrations. The error bars depict standard error. The observed concentrations ranged in back-transformed values of approximately 75 μg L^{-1} to 500 μg L^{-1}.

640