

1 **Phosphorus Fluxes in a Restored Carolina Bay Wetland Following Eight Years of Restoration**

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34 **ABSTRACT**

35 Restoring wetlands on agricultural land can release soil phosphorus (P) to surface waters.
36 Phosphorus is a limiting nutrient in many freshwater systems, thus restricting its release will
37 improve surface water quality by preventing algal blooms. A P balance was used to examine how
38 P was cycling in a Carolina Bay wetland eight years after restoration from prior-drained
39 agricultural land. The change in soil P was evaluated between archived samples taken at
40 restoration (2005), and eight years after restoration (2013). Measured P fluxes included
41 atmospheric deposition, plant uptake, and loss to surface water outflow. The soil total P pool at
42 the time of restoration was 810 kg P ha⁻¹. No significant ($\alpha=0.05$) decrease in the soil P pool was
43 observed over the eight years. Atmospheric deposition contributed 1.0 kg P ha⁻¹ yr⁻¹, plants
44 incorporated 3.3 P ha⁻¹ yr⁻¹ into woody biomass and 0.4 kg P ha⁻¹ yr⁻¹ as forest floor litter, and
45 0.2 kg P ha⁻¹ yr⁻¹ was lost to surface waters draining the wetland. Because the loss of P to surface
46 waters was small, and because runoff water concentrations of P declined through this period of
47 study to concentrations below those likely to cause eutrophication (< 0.1 mg L⁻¹), we concluded
48 that the wetland was not contributing to the degradation of surface water quality of nearby
49 streams following restoration. Further, isolated wetlands such as that studied may be promising
50 sites for future wetland mitigation projects due to limited impacts on surface water quality.

51 **KEY WORDS**

52 Wetland restoration; water quality; isolated wetlands; converted wetlands; agricultural land

53 **INTRODUCTION**

54 Over 50% of the original wetlands in the conterminous 48 states of the U.S. were drained
55 primarily for food production between 1780 and 1980 (Dahl and Allord 1996). Since 1977,
56 federal and state programs have been enacted to reverse the loss by restoring drained areas to
57 their original wetland condition, frequently by plugging or filling drainage ditches. Wetland
58 restoration is accomplished, in part, to improve water quality. However, in cases where wetlands
59 are restored from agricultural land that is high in phosphorus (P) from years of fertilization,
60 saturated and reduced soil conditions cause P to be released from the newly flooded wetland
61 contributing to eutrophication of nearby surface waters. Between 1997 and 2001 there was an
62 estimated annual net gain of 13,400 ha of wetlands nationally due to restoration of agricultural
63 fields, while between 2001 and 2003 the annual net gain more than doubled from previous
64 periods (USDA-NRCS 2013). Based on estimates from the North Carolina Division of
65 Mitigation Services, approximately two-thirds of restored wetlands in North Carolina originated
66 from drained and fertilized agricultural lands – equivalent to approximately 1,500 ha since 1999
67 (Smith 2011). Given that wetland restoration is increasing in the U.S., in part to improve water
68 quality, it is critical to determine whether restoration will contribute to pollution of P-sensitive
69 watersheds.

70 Phosphorus dissolution is the process in which P absorbed onto soil solids is released into the
71 soil solution (Moorberg et al 2015), making it susceptible to be lost from the soil to leaching,
72 runoff, or release to ponded water (Aldous et al 2005; Duff et al 2009; Ardon et al 2010).

73 Phosphorus dissolution from wetlands restored from agriculture has been observed around the

74 world. Recent studies of P dissolution have been done on peat soils in the Netherlands (Van Dijk
75 et al 2004), soils used for dairy production in Florida (Pant and Reddy 2003), restored lake fringe
76 in Oregon (Aldous et al 2007; Duff et al 2009), and agricultural soils in the North Carolina
77 Coastal Plain (Ardon et al 2010), and a Carolina Bay complex in North Carolina (Bruland et al
78 2003). These studies have shown that P dissolution is largely driven by iron (Fe) reduction
79 processes (Reddy and DeLaune 2008), along with other mechanisms including ligand exchange
80 (Earl et al 1979; Lopez-Hernandez et al 1986; Violante et al 1991; Gerke 1992), P mineralization
81 from drying and rewetting cycles (Song et al 2007), changes in pH (Jackson 1964;
82 Ponnampereuma 1972; Stumm and Morgan 1981), and increased P diffusion (Turner and Gilliam
83 1974a; Turner and Gilliam 1974b).

84 To ensure that wetland restoration and management practices do not contribute to pollution of
85 nutrient sensitive streams, a better understanding of P fluxes within and out of wetlands restored
86 from agricultural land is needed to identify potential management strategies that will reduce P
87 loss. This study focused on a previously cultivated Carolina Bay wetland, known as Juniper Bay,
88 that was restored as a wetland and then monitored for P in drainage waters in subsequent years
89 (Vepraskas et al 2010; Moorberg et al 2015; Moorberg et al 2017). The objectives of this study
90 were to estimate a P budget for a wetland restored from an agricultural field by determining P
91 fluxes for soil storage, atmospheric input, and loss from drainage water and estimating plant P
92 uptake.

93 **MATERIALS AND METHODS**

94 Juniper Bay is located in Robeson County, NC approximately 10 km south of Lumberton, NC
95 (34°30'30"N 79°01'30"W). In 1999, the North Carolina Department of Transportation purchased
96 this drained Carolina Bay wetland to mitigate the destruction of nearby wetlands caused by

97 highway construction (Ewing 2003). The Bay is oval-shaped, oriented lengthwise along a
98 northwest-southeast transect, and is virtually flat with an area of 291 ha. Soils in Juniper Bay
99 include approximately 60% (186 ha) mineral soils (Leon sand; sandy, siliceous, thermic Aeric
100 Alaquods, USDA Soil Taxonomy; (Soil Survey Staff 1999) primarily at the edges (Figure 1, SC
101 and SS mapping units), with organic soils (Ponzer muck; loamy, mixed, dysic, thermic Terric
102 Haplosaprists, USDA Soil Taxonomy; (Soil Survey Staff 1999), occupying the remainder (105
103 ha) at the center (Figure 1, OC and OS mapping units). This Bay was drained for agriculture
104 beginning in 1971 by excavating a perimeter ditch around the edge of the Bay, and installing
105 primary and secondary ditches within the Bay to facilitate drainage into a single surface water
106 outlet on the southwestern edge of the wetland (Vepraskas et al 2005). Juniper Bay was fertilized
107 and limed annually to meet soil-test recommendations. It remained in crop production until 2001.
108 Preliminary restoration efforts began in June 2003, and wetland hydrology was restored in 2005
109 by filling primary ditches and plugging tertiary ditches, leaving only the perimeter ditch intact.
110 That perimeter ditch drains into one outlet on the southwest side of the Bay.

111 A P-balance was developed to better understand the nature and relationships of P fluxes in and
112 out of Juniper Bay following restoration. The budget includes P inputs that are “new” sources of
113 P going into the soil, P outputs that are losses of P from the soil, and an error term that is the
114 remaining difference between inputs and outputs and/or error or fluxes that have gone
115 unrecognized. We hypothesized that atmospheric deposition (P_{ATM}) is a major mechanism
116 adding new P into the Bay, but groundwater inflow (P_{GI}) could also be contributing P. Major
117 ways for P to be removed from the soils in Juniper Bay include plant uptake (P_{PL}), groundwater
118 outflow (P_{GO}) and surface water outflow (P_{SO}). Previous work by Pati (2006) showed that the
119 perimeter ditch would intercept most groundwater inflow into the Bay, thus transforming P_{GI} into

120 P_{SO} which would drain out of the Bay through the outflow structure. In addition, Pati (2006)
 121 showed that the groundwater outflow component would be intercepted by the perimeter ditch as
 122 well, as long as the water levels in the ditch were managed to stay below a critical elevation.
 123 Such management of the perimeter ditch is currently practiced so that groundwater outflow from
 124 the Bay should be small. Huffman et al. (2007) estimated the net flow of ground and surface
 125 water into the Bay from the surrounding landscape was equivalent to 125 mm during the wet
 126 months of 2004, with inflows entering the perimeter ditch on the NW, NE, and SE sides of the
 127 Bay, and groundwater outflows exiting on the SW side of the Bay. The impact of the perimeter
 128 ditch is such that the terms for P_{GO}, P_{SO}, and P_{GI} were combined with the assumption that
 129 contributions from groundwater inflow are minimal, and that surface water outflow is primarily
 130 from drainage from Juniper Bay. This assumption was tested and validated, as described in the
 131 supplementary material (SI Table 1 and SI Table 2; SI Figure 1, SI Figure 2, SI Figure 3, and SI
 132 Figure 4)

133 The P balance for Juniper Bay can be written with the defined inputs and outputs as:

$$\Delta P_{\text{soil}} = P_{\text{ATM}} - P_{\text{PL}} - [P_{\text{GO}} + P_{\text{SO}} - P_{\text{GI}}] \quad [1]$$

134 For simplicity, we combined the components P_{GO}, P_{SO} and P_{GI} into one term called P_{OUTFLOW}
 135 which was measured collectively at the outflow structure. The modified P balance used for this
 136 study is:

$$\Delta P_{\text{soil}} = P_{\text{ATM}} - P_{\text{PL}} - P_{\text{OUTFLOW}} \pm E \quad [2]$$

137 The volume of soil considered for Juniper Bay has a horizontal area defined by the perimeter
 138 ditch (Figure 1B) with the soil depth starting at the soil surface and extending to a depth of 100

139 cm. The 100 cm depth was selected because previous work showed that the P increases from
140 agricultural applications in Juniper Bay were not observed below 100 cm (Ewing 2003).

141 The change in the soil P pool was determined by measuring total phosphorus (TP_{soil}) in archived
142 soil samples collected prior to restoration in 2005 and comparing those values with TP_{soil} found
143 in samples extracted from the same locations eight years after restoration of wetland hydrology
144 in 2013. The TP_{soil} concentrations for time-zero (2005) were determined from two groups of
145 archived soil samples. Prior to restoration, soils were sampled in 2000 from 48 soil pit locations
146 to a depth of 100 cm (Figure 1B) by Ewing et al. (2012), and in 2004 on a grid of 700 locations
147 across the Bay. Five samples were collected at each of the 700 grid sampling locations using a
148 2.2 cm diameter soil push probe at each sampling location to a depth of 30 cm. The samples were
149 separated into 0-15 cm and 15-30 cm depth increments, then composited for subsequent analysis.

150 It was assumed that P concentrations in the subsoil (30-100 cm) had not changed between 2000
151 and restoration in 2005 based on the observations made by Ewing et al. (2012) which noted that
152 subsoil P concentrations (4-7 g m⁻³) were comparable to those found in nearby reference
153 Carolina Bay wetlands (1-3 g m⁻³). Since Juniper Bay remained artificially drained from 2000 to
154 2004, soil P should have remained immobile prior to restoration.

155 For each archived soil sample location, GPS coordinates were recorded in 2005 at the time of
156 sampling, thus allowing new samples to be collected from the same locations. For the 0-15 cm
157 and 15-30 cm depths 138 locations of the 700 total were selected for re-sampling and analysis
158 using an area-weighted, stratified random sampling scheme (Figure 1B). The samples were
159 collected as described for the original 2004 sampling. The samples were separated into four
160 strata based on the soil mapping units developed for restoration of Juniper Bay during the North
161 Carolina Department of Transportation soil survey. All archived surface and subsoil sites were

162 located using GPS receivers with a wide area augmentation system correction and 2-5 m
163 accuracy.

164 The study by Ewing et al. (2012) included 48 soil pit locations. These locations consisted of 24
165 pairs of pits - one at the crest (middle) of the fieldlet, and one adjacent to the ditch. They
166 observed large amounts of disturbance in the ditch pits due to maintenance and dredging of the
167 drainage ditches during agricultural production; therefore, only the crest pits were used in this
168 study. Also, Ewing et al. (2012) studied five pits from soils with histic epipedons at the transition
169 from mineral to organic soils. Because these histic soils represented a small area of the Bay, they
170 were also omitted from this study. The remaining 19 soil pits used in this study are shown in
171 Figure 1B. The 2013 soil samples were extracted using a 7.6 cm diameter soil bucket auger for
172 all three depths.

173 All soil samples were submitted to the North Carolina Department of Agriculture Soil Testing
174 Service for analysis of extractable P by the Mehlich-3 method (Mehlich 1984) with P
175 concentrations determined using Inductively Coupled Plasma Mass Spectroscopy. Soil TP
176 analysis was performed on 25% of the re-sampled 0-15 cm and 15-30 cm depth soil samples that
177 were selected at random within each stratum (Figure 1B). Soil TP analysis was also performed
178 on four representative horizons from each pit location (Figure 1B). Soil TP was determined by
179 performing a nitric-perchloric acid digestion (Carter 1993) which was analyzed by the North
180 Carolina State University Environmental and Agricultural Testing Service (Raleigh, North
181 Carolina, USA) where P concentrations were determined using Inductively Coupled Plasma
182 Mass Spectroscopy. The TP_{soil} determined on a mass per mass basis were re-expressed as mass P
183 per volume of soil for inclusion into the P-balance. Bulk densities reported by Ewing et al.
184 (2012) for the pre-restoration samples at all depths were used to convert the mass of soil to its

185 equivalent volume. Bulk density was determined again for the 2013 samples for the 0-15 cm and
186 15-30 cm depths using the core method (Grossman and Reinsch 2002). Samples were collected
187 in triplicate at each of the 19 soil pit locations and averaged across the four soil mapping units
188 for the 0-15 cm and 15-30 cm depths. Bulk densities for the 30-100 cm depths were assumed to
189 be the same as the pre-restoration values as reported by Ewing et al. (2012).

190 Atmospheric deposition of P was monitored from May 2012 through June 2013 at three locations
191 within the wetland (Figure 1A), as described by Kreiser (2003). Samplers were installed adjacent
192 to existing rain gauges using a bulk rain water collection apparatus (SI Figure 5) modeled after
193 Likens et al. (1967) and Johnson and Swank (1973). Samples were collected every two to four
194 weeks and acidified for preservation. Samples were submitted to the North Carolina State
195 University Environmental and Agricultural Testing Service (Raleigh, North Carolina, USA) for
196 determination of dissolved reactive P (DRP) and dissolved total P (DTP). Dissolved reactive P
197 was measured colorimetrically using a multichannel QuikChem 8000 (Lachat Instruments,
198 Milwaukee, WI, USA) using the method of Prokopy and Wendt (1994). Dissolved total P was
199 analyzed colorimetrically simultaneously on the same instrument and the method described by
200 (Liao 2001). The average concentration of DTP for this time period, along with historic rainfall
201 data collected on site were used to estimate P_{atm} from 2005 to 2013 over the entire 291 ha area of
202 the wetland.

203 Phosphorus uptake and accumulation by trees was estimated for the entire area of Juniper Bay.
204 The North Carolina Department of Environment and Natural Resources (now the North Carolina
205 Department of Environmental Quality, NCDEQ) planted wetland tree saplings throughout
206 Juniper Bay at the time of restoration (NCDEQ 2010). Between 2005 and 2010, NCDEQ (2010)
207 established and maintained 19 vegetation plots for wetland mitigation purposes at Juniper Bay.

208 Those 10 m by 10 m plots were located and expanded by 20 m on all sides to create 30 m x 30 m
209 plots for this tree survey, as shown in Figure 1A (plots not drawn to scale). Tree species, height,
210 and diameter at breast height (DBH) were recorded for all trees greater than 10 cm DBH within
211 each vegetation plot. Wood biomass was then estimated for each tree using allometric equations
212 from Gonzalez-Benecke (2011) for loblolly pine (*Pinus taeda* L.) and pond pine (*Pinus serotina*
213 Michx.), and allometric equations from Schroeder et al. (1997) and Jenkins et al. (2003) for all
214 other species. Biomass P content was estimated for all species using P concentrations presented
215 by Bedford et al. (1999). The total plot woody biomass per-hectare (kg ha^{-1}) and woody biomass
216 P (kg P ha^{-1}) was determined by summing all of the tree biomass and biomass P within each plot,
217 and dividing by the plot area.

218 Plant litter (the fibric, Oi soil horizon) samples were collected in October of 2014 from eight
219 randomly selected vegetation plots. A 1 m by 1 m square PVC frame was laid in the center of
220 each plot. Five large nails were driven into the ground until the nail head was at the litter surface
221 at each of the four corners and one at the center. The litter was then collected for analysis. The
222 heights of each nail above the soil surface were measured and averaged to determine the average
223 depth of plant litter per square meter.

224 The litter was dried in an oven at 70°C for four days, then weighed to determine total litter
225 biomass. Subsamples were ground and analyzed by the Environmental and Agricultural Testing
226 Laboratory at North Carolina State University (Raleigh, North Carolina, USA) for C and P
227 analysis. Carbon was analyzed using a PerkinElmer model 2400 CHN elemental analyzer.
228 Phosphorus was determined with a dry-ash method based on the method described by Jones and
229 Case (1990). The percent P by weight (% w/w) for the subsamples was multiplied by the litter
230 dry weight per square meter (g m^{-2}) to estimate the mass of P per square meter (g P m^{-2}) for each

231 site. This value was then converted to kg P ha^{-1} , which represents the amount of P that has
232 accumulated in the litter over the eight years since restoration.

233 The perimeter ditch surrounding Juniper Bay drains into a single surface water outflow structure
234 at the edge of the Bay (Figure 1A). Samples from the drainage outlet were taken four times daily
235 from 2010 to 2013 using a Teledyne ISCO automatic water sampler (Teledyne ISCO, Lincoln,
236 Nebraska, USA) and composited into one sample. These samples were collected approximately
237 every 14 days. From 2005 to 2010, manually collected samples (1 L volume) were collected
238 from the center of the channel at the outflow monthly using a bottle attached to a pole. All water
239 samples were acidified for preservation and submitted to the North Carolina State University
240 Environmental and Agricultural Testing Service (Raleigh, North Carolina, USA) for DRP and
241 DTP analysis. Dissolved reactive P was measured colorimetrically using a multichannel
242 QuikChem 8000 (Lachat Instruments, Milwaukee, WI, USA) using the method of Prokopy and
243 Wendt (1994). Dissolved total P was analyzed colorimetrically simultaneously on the same
244 instrument and the method described by (Liao 2001). Only DRP was measured on the grab
245 samples (2005-2010), while both DRP and DTP were analyzed for the daily samples (2010-
246 2013). Organic P (difference between DTP and DRP) was not determined from 2005-2010 and
247 was assumed to contribute to the error term in the P balance as an un-accounted loss. A subset of
248 samples was also analyzed for total P, but no significant difference between total P and DTP was
249 observed. This indicated that particulate P was not present at this site in measurable amounts, so
250 DTP was used for calculating P_{outflow} instead of total P.

251 Discharge rates were measured from December 2010 through 2013. Surface outflow in the
252 perimeter ditch was measured at the main outlet using dual compound weirs installed in 2001 as
253 described by Vepraskas et al. (2005) Compound weirs consist of a V-notch cut into the center of

254 the crest of a larger rectangular notch. Discharge is calculated using two different equations
255 depending on if the discharge is contained in the V-notch or rectangular portion of the weir.
256 During high flow events the discharge was calculated as follows (United States Bureau of
257 Reclamation 2001):

$$Q = 3.9h_1^{1.72} - 1.5 + 3.3Lh_2^{1.5} \quad [3]$$

258 where Q is the discharge in ft³/s, h₁ is the head above the point of the V-notch in ft, L is the
259 combined length of the horizontal portions of the weir in ft, and h₂ is the head above the
260 horizontal crest in ft. If flow was confined to only the V-notch portion of the weir, then the
261 standard V-notch equation was used (United States Bureau of Reclamation 2001):

$$Q = 2.49 \times h_1^{2.48} \quad [4]$$

262 Using the same variables identified above. The stage height was determined using two pressure
263 transducers (HOBO U20L-04 Water Level pressure transducers, Onset Computer Corp, Bourne,
264 MA, USA), one installed below the weir and the other above. This height was used to determine
265 which part of the weir the water was in, and thus determine which equation to use. The pressure
266 transducer data was recorded using a Campbell Scientific CR-10X data logger (Campbell
267 Scientific, Logan, UT, USA).

268 Surface water discharge was estimated prior to December 2010 using a monthly water balance.
269 Rainfall was measured on site at three rainfall stations (SI Table 4). Evapotranspiration rates
270 from the MODIS Land Subsets Oak Ridge National Laboratory Distributed Active Archive
271 Center (ORNL DAAC, 2011) were used from January 2005 through December 2013. The
272 MODIS Land Subset ET is remotely sensed ET with a 500 m resolution that is determined from

273 leaf area index (LAI) and radiation at the earth's surface. That dataset provides total ET over 8
274 days. To estimate monthly ET, the 8-day ET values were divided by eight to estimate the daily
275 ET value on the day it was reported. Missing daily ET values were then interpolated using
276 MatLab (MathWorks, Natick, MA, USA) and summed for each month to estimate total monthly
277 ET from January 2005 to December 2009. Evapotranspiration from January 2010 to November
278 2010 was estimated using the Thornthwaite method (Thornthwaite 1948) and monthly mean
279 temperature data from the Lumberton Regional Airport (NOAA NCDC 2013) approximately 11
280 km northwest of Juniper Bay. The total amount of P lost in the drainage water was calculated by
281 multiplying P concentrations in the drainage water by the volume of drainage water leaving
282 through the outflow. This calculation was performed on a daily basis from December 2010 to
283 2013, and on a monthly basis for 2005 through November 2010.

284 The ΔP_{soil} was determined on an annual basis over the 8-year period since the wetland was
285 restored (2005 to 2013). The error (E) term in Equation 2, based on measured fluxes, was
286 calculated as the remainder term between the soil ΔP_{soil} and flux ΔP .

287 Statistical analysis was performed on the soil total P data using PROC GLIMMIX in SAS 9.3
288 (SAS Institute Inc., Cary, NC, USA) with a gamma distribution. The LSMeans presented were
289 back-transformed using an "ilink" command. Mehlich-3 extractable P data were analyzed with a
290 natural log transformation using PROC MIXED in SAS 9.3. The LSMeans reported were back-
291 transformed using a procedure described by Jørgensen and Pedersen (2013). Confidence
292 intervals were corrected for multiple comparisons using a Tukey adjustment. A t-test was
293 performed in SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA) to test for differences
294 in woody biomass and biomass P content between the mineral and organic soils. Summary
295 statistics were determined for P_{atm} using SigmaPlot 12.5.

296 **RESULTS**

297 Total soil P concentrations in 2005 and 2013 are summarized in Table 1 and reported as kg P ha⁻¹
298 basis in SI Table 3. No difference in soil TP was detected between 2005 and 2013 (p=0.42) for
299 the entire Bay to a depth of 1 m. There was also no significant difference between years at any
300 depth for either the mineral or organic soils. Likewise, there was no significant change in
301 Mehlich-3 extractable P between 2005 and 2013 (SI Table 4, SI Table 5). The organic soils at
302 Juniper Bay had higher (p<0.0001) concentrations of TP (0.131 kg m⁻³, SE 0.013) than the
303 mineral soils (0.072 kg m⁻³, SE 0.005) across all three depths and both sampling years. The TP
304 concentrations were highest at the surface for both mineral and organic soils in 2005 and 2013
305 (p<0.0001). The sum of total soil P on a per-hectare basis to a depth of 1 m in 2005 was 1,094 kg
306 P ha⁻¹ for the organic soils which represent 105 ha (40% of the Bay) and 642 kg P ha⁻¹ for the
307 mineral soils which represent 186 ha (60% of the Bay). Thus, the total pool of total soil P in
308 2005, calculated as an area-weighted average, was 804 kg P ha⁻¹. In comparison the total pool in
309 2013 was 758 kg P ha⁻¹ (1,008 kg P ha⁻¹ and 592 kg P ha⁻¹ for the organic and mineral soils,
310 respectively), though again, the differences between total soil P concentrations were not
311 significant.

312 Results from a separate study examining P fractions at Juniper Bay compared to two unfarmed
313 reference Carolina Bays are presented in the Supplemental Information (SI Table 10). The
314 history of agriculture and fertilization at Juniper Bay increased both inorganic and organic P
315 relative to the reference Carolina Bays, though the largest increases were to inorganic P.

316 Rainfall P concentrations during the duration of the study averaged 0.11 mg DTP L⁻¹ (SE 0.02).

317 There was no significant difference in P among the three stations. The average concentration of

318 rainfall DTP from 2005 to 2012 was assumed to be equal to the 0.11 mg DTP L⁻¹ observed in
319 this study. That concentration and daily rainfall data for Juniper Bay were used, along with the
320 total area of the Bay, to estimate the P_{atm} following restoration in 2005 – a total of 8 kg ha⁻¹ over
321 eight years, or 1.0 kg ha⁻¹ yr⁻¹. Monthly rainfall is summarized in SI Table 4. Estimated monthly
322 P_{atm} is summarized on a per-hectare basis in Table 2.

323 There was no significant difference in estimated woody biomass or estimated woody biomass P
324 between soil types (mineral versus organic). The average values across all plots were used for
325 further calculations. The estimated average woody biomass was 29,300 kg ha⁻¹ (SE 5,400), and
326 the estimated average woody biomass P was 26.6 kg P ha⁻¹ (SE 4.9). This translates to 3.33 kg P
327 ha⁻¹ yr⁻¹ over the eight years following restoration. The plant litter biomass and P contents are
328 shown in Table 3. The average accumulation of P into the litter layer was 3.2 kg P ha⁻¹ (SE 0.7),
329 or 0.4 kg P ha⁻¹ yr⁻¹ (SE 0.1).

330 Discharge rates from Juniper Bay were estimated prior to December 2010 based on a simple
331 water balance as the difference between monthly rainfall (SI Table 4) and monthly ET (SI Table
332 5). Rainfall was compared to normal values for the AgACIS WETS table for the Lumberton
333 Regional Airport which had an observation period of 1971-2000 (USDA-NRCS 2021). Less than
334 normal rainfall was observed for 2005, 2006, 2007, 2011 and 2012, while 2008-2010 had normal
335 rainfall. Evapotranspiration rates are summarized in SI Table 5. Discharge from the single
336 outflow at Juniper Bay was measured directly starting in December 2010. Monthly discharge
337 rates are summarized in SI Table 6. For months where the estimated discharge was negative
338 (ET>rainfall) the discharge was assumed to be 0 mm.

339 The concentration of DRP over time at the Juniper Bay outflow is shown in Figure 2. The
340 concentration of DRP increased following restoration in 2005 and remained at elevated
341 concentrations until 2010. Following 2010 the concentrations declined to pre-restoration levels.
342 The P discharge, estimated on a monthly basis for 2005-2010 and a daily basis for 2011-2012,
343 averaged $0.2 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ during the eight years following restoration. Exports of P are
344 summarized in SI Table 9.

345 The P balance for Juniper Bay is summarized in Figure 3. The main flux of P entering the Bay
346 during this study was from the atmosphere at $1.0 \text{ kg ha}^{-1} \text{ yr}^{-1}$. Plant uptake into woody biomass
347 was the largest P flux out of the soil and was estimated at $3.3 \text{ kg P ha}^{-1} \text{ yr}^{-1}$. Phosphorus that had
348 accumulated in the forest floor litter was estimated at $0.4 \text{ kg P ha}^{-1} \text{ yr}^{-1}$. Phosphorus leaving the
349 site through the drainage water was estimated at $0.2 \text{ kg P ha}^{-1} \text{ yr}^{-1}$. This leaves an error term of -
350 $2.9 \text{ kg P ha}^{-1} \text{ yr}^{-1}$.

351 **DISCUSSION**

352 The primary focus of this study was to determine if Juniper Bay has been, is currently, or will be
353 a source of P for downstream surface waters following restoration of its prior-drained
354 agricultural land. The increase in soil P over 30 years of agricultural fertilization did lead to soil
355 P concentrations significantly higher than un-farmed reference Carolina Bays (Ewing et al 2012)
356 and a total P pool of 804 kg P ha^{-1} at the time of restoration in 2005. A separate study in 2010
357 presented in the SI showed those increases occurred in both the mineral and organic P fractions
358 of both the mineral and organic soils with the largest proportional increases occurring in the
359 inorganic P. For this P balance study comparing pre- and post-restoration soil samples there was

360 a nominal decrease in total soil P concentrations. However, those differences were not significant
361 so we must assume that there was no change in the soil P pool following restoration.

362 A P balance was estimated in order to better understand P fluxes at Juniper Bay, and to guide
363 future management. The main flux of P into the Bay was P_{atm} . Atmospheric deposition of P was
364 small, at $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$, which is similar to the median annual deposition of TP in North America
365 of 3.2 kg ha^{-1} reported by Tipping et al. (2014). The concentration of DTP in the rainwater was
366 also approximately the same concentration as in the drainage water. Because the runoff ratio
367 (ratio of runoff to total rainfall) of Juniper Bay is very small, very little of that rain (and P)
368 reached the outflow structure. The flux of P out of Juniper Bay in the drainage water was under
369 $0.2 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ during the eight years following restoration. The largest P loss was due to plant
370 uptake. The average annual incorporation of P into woody biomass was $3.3 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ in
371 addition to $0.4 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ accumulating in the litter. This flux of P from the soil and into
372 woody biomass and plant litter should slow any potential release of P to drainage waters.

373 Our estimated error term, $E (-2.9 \text{ kg P ha}^{-1} \text{ yr}^{-1})$ was calculated assuming ΔP_{soil} is $0 \text{ kg ha}^{-1} \text{ yr}^{-1}$
374 due to finding no significant difference between soil total P between 2005 and 2013. The
375 magnitude of the soil P pool, and the similarly large error in total soil P are an order of
376 magnitude higher than the fluxes reported. This is the most likely contributing factor towards the
377 calculated error in this P balance. A more intensive soil sampling approach may reduce those
378 error terms in total soil P. There are other potential sources of error as well. One is having only
379 DRP and not DTP measurements of the drainage water prior to 2010. The organic P missed may
380 account for some P flux out of the wetland. However, the measured flux of P in the drainage
381 water was still very small, smaller than the soil P pool by three to four orders of magnitude. We
382 note that we did not measure P concentrations of woody tissue in this study, nor did we conduct

383 repeated sampling of litterfall. Such changes would likely have improved our accuracy of P lost
384 to plant uptake or litter accumulation. Further, plant uptake is likely larger than was predicted
385 due to P taken up by small trees (<10 cm DBH), shrubs, and herbaceous plants, which were not
386 measured in this study.

387 The agreement of measured runoff volumes at the outflow with runoff predicted with a water
388 balance suggest that losses or gains of water from the surrounding landscape are relatively
389 minimal (see supplemental information). This is further evidenced by the small hydrologic
390 gradients in four transects around the Bay (SI Table 1), and the resulting relatively slow
391 porewater velocities (SI Table 2).

392 While post-restoration concentrations of P in the Juniper Bay drainage water did depict a small
393 release of P out of Juniper Bay, concentrations have since declined to pre-restoration levels (\leq
394 0.1 mg P L^{-1}) within five years of restoration (Figure 2). Phosphorus concentrations above 0.1
395 mg L^{-1} would be expected to contribute to eutrophication in freshwater systems (Correll 1998).
396 The concentration of P in the drainage water was also equal to P concentrations found in the
397 rainwater. In addition, the P that has left Juniper Bay through the outflow since restoration
398 accounts for approximately 0.2% of the total pool of P at the site in 2005. Because of the low
399 concentrations of P in the drainage water, and the low magnitude of P losses to the drainage
400 water relative to the total P pool, P export from Juniper Bay to surface waters is not expected to
401 be a major concern in the future. Bruland et al. (2003) determined that in a restored Carolina Bay
402 wetland complex the export of soluble reactive P and total P from the restored wetland was less
403 than that of an actively farmed wetland, thus concluding that wetland restoration after just two
404 years resulted in a net improvement to water quality. Concentrations of P following restoration
405 did eventually decrease to concentrations observed following agricultural production before

406 wetland restoration. Plant uptake may also reduce the amount of plant available P – the P
407 fraction most easily exported. However, P loss due to plant uptake alone will likely take decades
408 to centuries to reduce soil P concentrations down to natural concentrations.

409 The results of this study indicate that while most of the residual soil P that was left over from
410 agricultural production is still in the Juniper Bay soils, it is not moving off site. This indicates
411 that Carolina Bays, like Juniper Bay, may make excellent potential sites for wetland restoration.
412 However, Carolina Bays that are drained by streams may be exceptions. Such wetlands would be
413 expected to have more P leaving the site through surface outflow because of higher hydraulic
414 gradients caused by the dissecting streams. The ideal areas for wetland restoration are closed
415 depressions that have precipitation as the main water source and evapotranspiration as the main
416 water loss.

417 In weighing the potential risks and benefits of restoring Carolina Bay wetlands that have been
418 used for production agriculture, it is important to consider the conversions between land uses for
419 these wetlands within the region. In a concurrent study, separate from that presented here,
420 Sullivan et al. (2017) inventoried Carolina Bays in Bladen County, North Carolina a
421 representative “Bay-dense” region within the southeastern Coastal Plain close to Robeson
422 County, North Carolina where the present study was conducted . They documented land-use
423 change from 1972 through 2010 using decadal Landsat imagery. They found that during that
424 time period, 43% of the Bays and 91% of the Bay area were associated with land-use change
425 between 1972 and 2010. In 1972, Bays were predominantly forested (79% by count), with
426 remaining Bays converted to agriculture or urban use prior to 1972. Land-use changes were
427 predominantly from forest to agriculture (46%) and agriculture to forest (37%). Conversion to
428 forest remained low from 1984 to 1991 and from 2000 to 2010, with a net loss in agricultural

429 land use of 2,085 and 1,457 ha, respectively. A surge in conversion from forest to agriculture
430 occurred between 1991 and 2000, which was surprising given the 1990 US Army Corps of
431 Engineers/Environmental Protection Agency agreement targeting no net wetland loss. From
432 1972 through 2010, there was an estimated net gain of 744 ha of Bay forest relative to Bay
433 agriculture. Sullivan et al. (2019) conducted a follow-up study assessing the risk of P export to
434 nearby or intersecting streams. They found that 1,360 Carolina Bays in Bladen County, North
435 Carolina representing 43% of the Bays and 80% of the total Bay area had streams that either
436 intersected the Bays or came within 15 m of the edge of a the Bays. These wetlands posed a risk
437 of P export depending on the land use or changes in land use of each Bay. Isolated wetlands
438 without a nearby or intersecting stream were determined to pose little risk of P export.

439 **SUMMARY & CONCLUSIONS**

440 The objective of this study was to create a P balance for a Carolina Bay restored from
441 agricultural land. Juniper Bay was restored by filling in primary ditches, plugging secondary
442 ditches, and maintaining the perimeter ditch surrounding the site. This resulted in a low hydraulic
443 gradient for drainage water. The soil P pool to a depth of 1 m was determined to be 804 kg P ha⁻¹
444 in 2005 and 742 kg P ha⁻¹ in 2013, though this difference was not significant. Phosphorus fluxes
445 into and out of Juniper Bay included a gain in P from atmospheric deposition, and losses of P to
446 surface water outflow and plant uptake. The error term depicts an unaccounted loss of 2.9 kg ha⁻¹
447 yr⁻¹. This is miniscule compared to the size of the P pool existing in the soil at Juniper bay and is
448 smaller than all standard errors reported for total soil P (SI Table 3). Phosphorus loss to surface
449 waters was minimal both in magnitude (0.2 kg P ha⁻¹ yr⁻¹) and in current concentrations
450 (approximately 0.1 mg P/L). The concentration of P exiting the Bay is approximately the same as

451 was observed in rainwater at Juniper Bay, and is not expected to contribute to eutrophication of
452 downstream surface waters.

453 The key takeaway from this study is Carolina Bay wetlands similar to Juniper Bay are promising
454 sites for wetland restorations due to the low risk of P contributions following restoration from
455 agricultural production. Carolina Bays are broad and nearly level, which results in very low
456 hydraulic gradients following restoration back to wetlands. This likely reduces movement of P to
457 nearby streams or drainageways. However, internal drainage ditches must be filled and/or
458 plugged during the restoration process. Carolina Bays that are deeply dissected by streams or
459 ditches may have sufficient hydraulic gradients to facilitate P transport, so restoration of such sites
460 must be done with care.

461 **DECLARATIONS**

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464 Carolina System, and by the US Department of Agriculture - Agriculture and Food Research
465 Initiative.

466 **Conflicts of Interest**

467 The authors have no conflicts of interest.

468 **Availability of Data and Material**

469 The datasets generated during and/or analyzed during the current study are available from the
470 corresponding author on reasonable request.

471 **Code Availability**

472 The code used during the current study is available via Moorberg (2014)

473 **Authors' Contributions**

474 CJM lead efforts for sample collection, sample analysis, data analysis, data visualization, and
475 writing. MJV was the principle investigator for both sources of funding, supervised the research
476 activities of CJM, and was a major contributor to writing the manuscript. JGW assisted with
477 sample collection for the archived soil samples. DDR assisted soil total P analysis. All authors
478 provided revisions to manuscript drafts and read and approved the final draft.

479 **Ethics Approval**

480 Not applicable

481 **Consent to Participate**
482 Not applicable
483 **Consent for Publication**
484 Not applicable

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- 629

630 TABLES

631 Table 1. Soil total P concentrations by soil type, depth, and year.

Soil	Depth	2005			2013		
		LSMean	SE	Significance	LSMean	SE	Significance
	cm	kg TP m ⁻³		a b c	kg TP m ⁻³		a b c
Mineral	15	0.122 ± 0.013		A A A	0.132 ± 0.015		A A A
Mineral	30	0.069 ± 0.008		A B A	0.058 ± 0.007		A B A
Mineral	100	0.051 ± 0.007		A B A	0.044 ± 0.006		A B A
Organic	15	0.257 ± 0.039		A A B	0.207 ± 0.032		A A B
Organic	30	0.118 ± 0.018		A B B	0.160 ± 0.025		A B B
Organic	100	0.076 ± 0.017		A B A	0.065 ± 0.015		A C A

632 ^aComparison of total P concentration between years for a given soil type and depth in column
633 ^bComparison of total P concentration between depths within a given year and soil type in column
634 ^cComparison of total P concentration between soil types within a given year at a given depth in
635 column
636 Means with the same letter not significantly different ($\alpha=0.05$).

637 Table 2. Monthly and Annual atmospheric P deposition (P_{atm} , kg TP ha⁻¹) between
 638 January 2005 and December 2012.

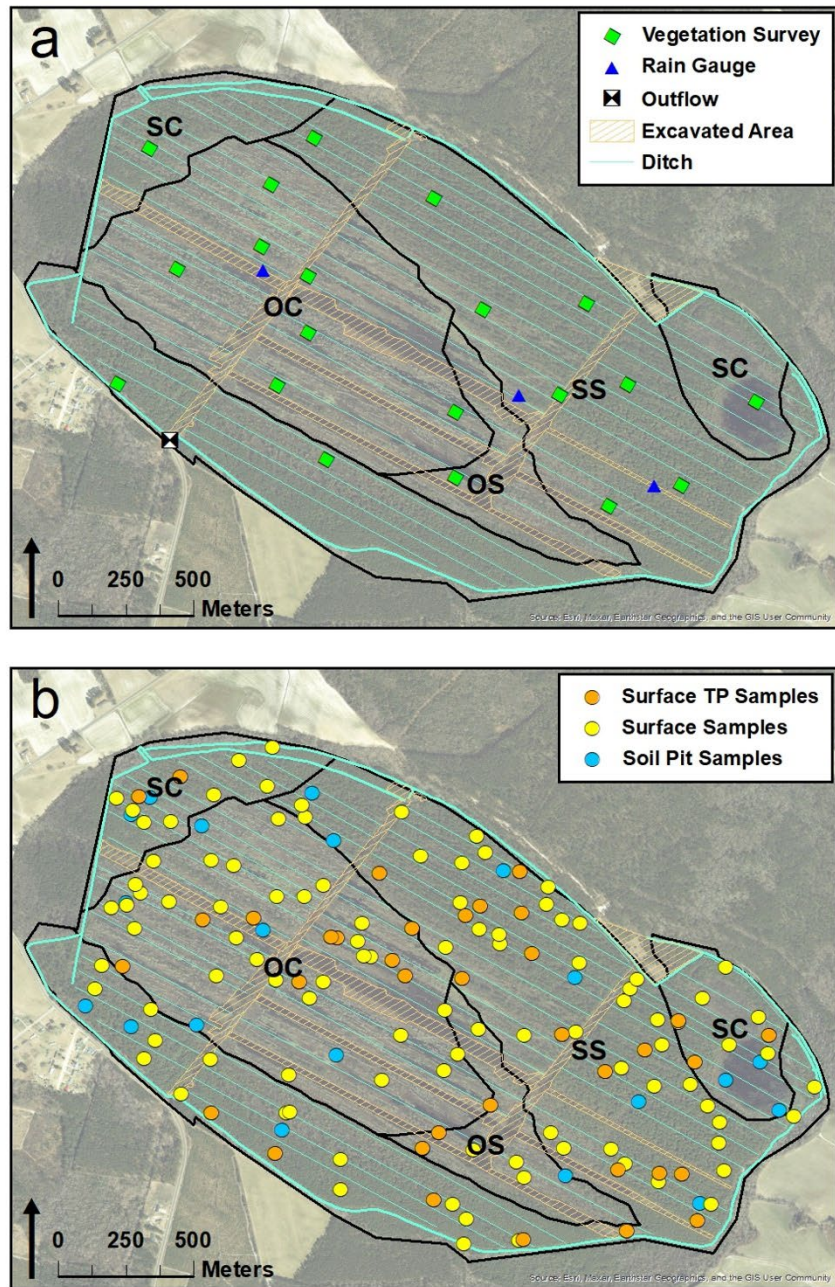
Month	2005 ^a	2006	2007	2008	2009	2010	2011	2012
Jan	0.056	0.077	0.018	0.079	0.035	0.090	0.037	0.077
Feb	0.046	0.078	0.048	0.116	0.047	0.116	0.133	0.077
Mar	0.054	0.015	0.040	0.104	0.093	0.089	0.112	0.101
Apr	0.055	0.047	0.070	0.101	0.030	0.019	0.067	0.059
May	0.041	0.137	0.045	0.081	0.262	0.085	0.102	0.169
Jun	0.053	0.146	0.092	0.078	0.127	0.186	0.041	0.114
Jul	0.126	0.122	0.015	0.096	0.111	0.277	0.099	0.099
Aug	0.015	0.154	0.066	0.179	0.162	0.082	0.198	0.236
Sep	0.037	0.029	0.012	0.229	0.009	0.212	0.101	0.090
Oct	0.067	0.019	0.048	0.022	0.075	0.034	0.061	0.033
Nov	0.090	0.097	0.002	0.128	0.194	0.035	0.090	0.000
Dec	0.054	0.079	0.113	0.080	0.165	0.064	0.020	0.077
Total	0.695	1.001	0.570	1.294	1.310	1.289	1.062	1.133

639 ^aRainfall data was acquired from a nearby weather station at the Lumberton, NC airport (NOAA
 640 NCDC 2013).

641 Table 3. Plant litter biomass and P content.

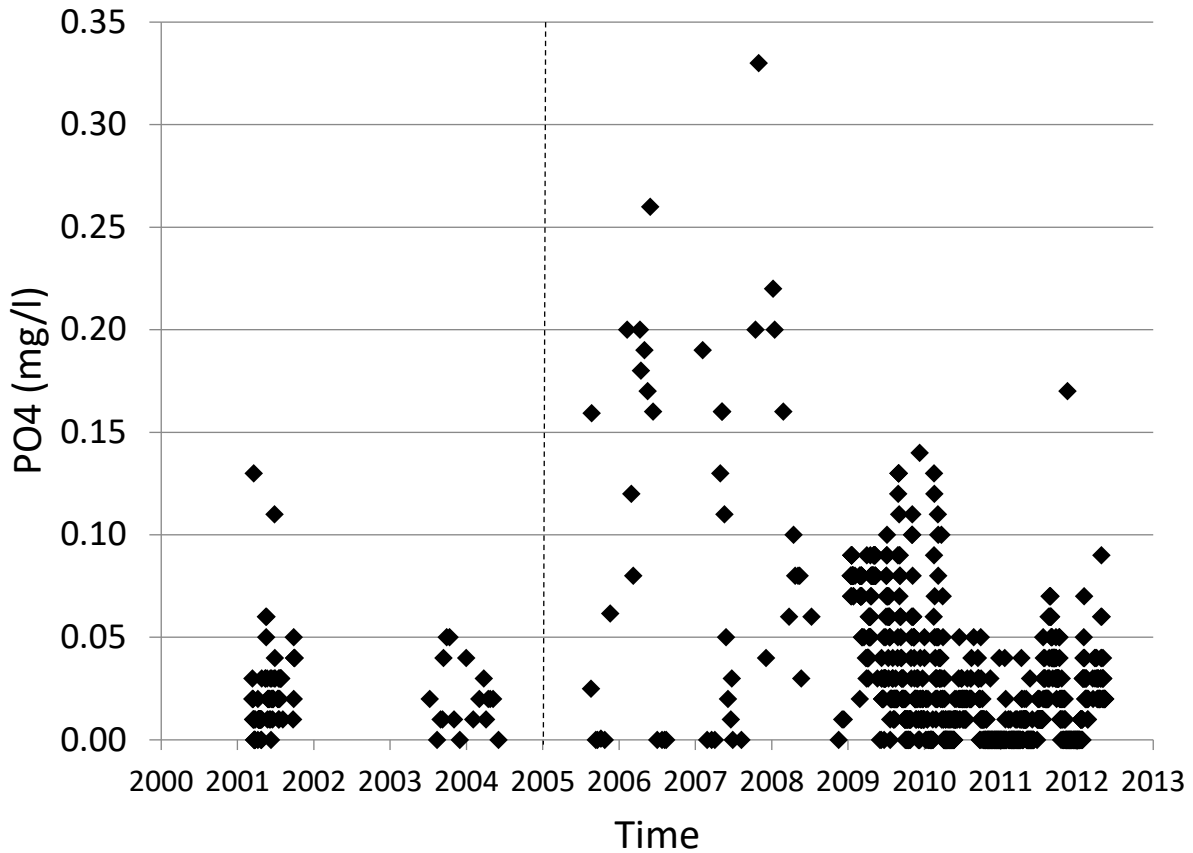
Plot	Dominant Trees Present	Average Litter Depth (cm)	Litter Dry Weight	P Concentration	Litter P
		cm	g	% (w/w)	kg P ha ⁻¹
1	Pine	4.50	850.6	0.08	7.1
8	Bald Cypress	2.60	337.7	0.09	3.0
9	Pine, Oak, Sweetgum	4.82	870.4	0.05	4.6
12	Bald Cypress, Pine, Willow	2.76	274.4	0.05	1.4
14	Pine, Bay	1.38	259.5	0.07	1.8
15	Bay, Bald Cypress	2.58	290.5	0.10	3.0
16	Oak	3.90	488.8	0.07	3.6
19	Bald Cypress	0.76	82.5	0.11	0.9
Average litter P					3.2±0.7

642



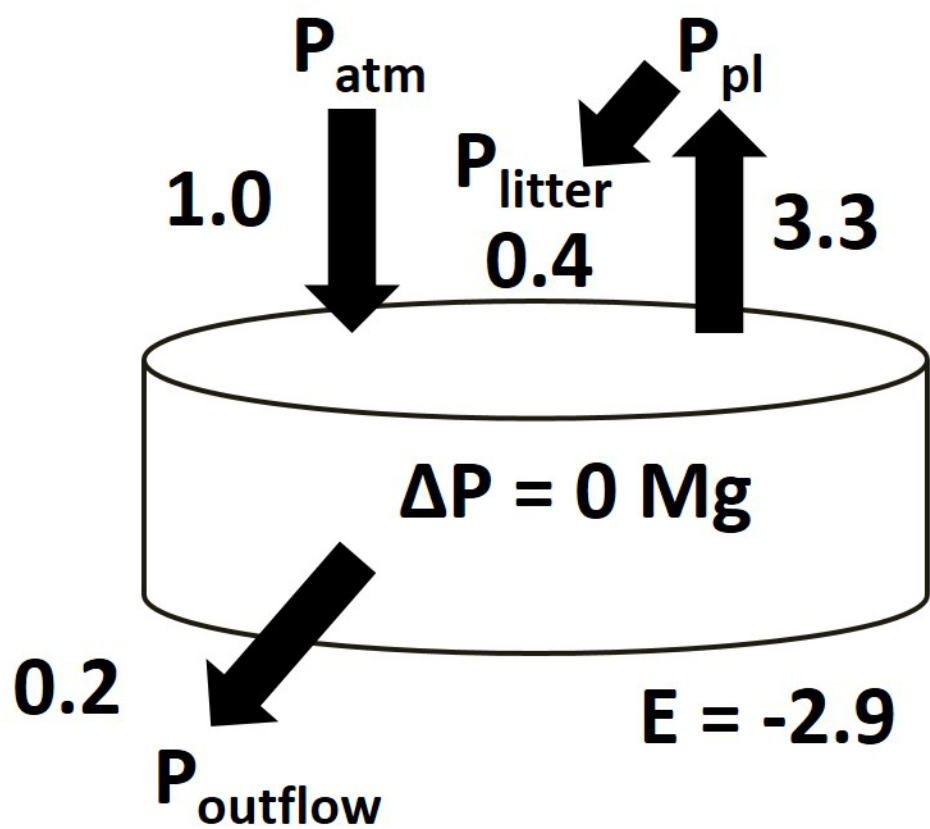
644

645 Figure 1. Maps of Juniper Bay depicting locations of a) previous and existing drainage
 646 ditches, perimeter ditch outflow, vegetation survey locations (not drawn to scale), rain
 647 gauge location, and mineral and organic soil distribution; and b) resampled surface soil
 648 locations for extractable and total P, and resampled soil pit locations. The mapping units
 649 depict four soil conditions, including sands over clayey subsoil (SC), sands over sandy
 650 subsoil (SS), organic soil over clayey subsoil (OC), and organic soil over sandy subsoil
 651 (OS). The scale of both maps is 1:12,000.



652

653 Figure 2. Concentration of dissolved reactive P at the Juniper Bay outflow over time.
 654 The wetland was restored in 2005 (dashed line), after which an increase in dissolved
 655 reactive P at the outflow was observed through approximately 2010. The dissolved
 656 reactive P concentrations declined to pre-restoration levels thereafter. Eutrophication
 657 would be expected at 0.10 mg dissolved reactive P per liter in freshwaters, assuming N is
 658 not limiting. Outflow concentrations only exceeded that concentration once after 2010.



659

660 Figure 3. Summary of the P balance for Juniper Bay from 2005-2013. The fluxes are
 661 reported as $\text{kg P ha}^{-1} \text{ yr}^{-1}$ and include change in total P (ΔP_{soil}), atmospheric deposition
 662 (P_{atm}), plant uptake (P_{pl}), plant litter (P_{litter}), surface water outflow (P_{outflow}), and
 663 error (E).

664 **LIST OF SUPPLEMENTAL TABLES**

665 SI Table 1. Water Balance Test of Hydrology Assumptions.

666 SI Table 2. Estimations of Flow for Each Transect and Sand Layer. Porewater velocity was
667 estimated from Hydraulic gradients and soil properties for the four piezometer transects at
668 Juniper Bay. The saturated conductivities used were reported by Pati (2006). Porosity was
669 estimated from average bulk densities reported by Ewing (2003). Hydraulic gradients were
670 calculated from measurements made on May 15, 2013. Flow rates were small overall, due to
671 both the small gradients present, and the low conductivities.

672 SI Table 3. Soil P content (kg P ha^{-1}) in 2005 and 2013.

673 SI Table 4. Changes in Mehlich-3 P.

674 SI Table 5. Mehlich-3 P content (kg P ha^{-1}) in 2005 and 2013.

675 SI Table 6. Monthly Rainfall from January 2005 to December 2012.

676 SI Table 7. Monthly ET between January 2005 and December 2012. Evapotranspiration was
677 measured remotely for 2005-2009, and estimated using the Thornthwaite equation in from 2010
678 to 2012.

679 SI Table 8. Monthly runoff between January 2005 and December 2012. Runoff was estimated
680 using a simple water balance for 2005-2010, and measured directly for 2011 and 2012. For
681 estimations of P, months with negative estimated runoff were assumed to have no runoff.

682 SI Table 9. Monthly P exports between January 2005 and December 2012. Export was estimated
683 as the product of runoff volume and P concentration in the runoff. For estimations of P, months
684 with negative estimated runoff were assumed to have no runoff.

685 SI Table 10. Summary of soil P fractions from Juniper Bay and two reference Carolina Bays.

686 **LIST OF SUPPLEMENTAL FIGURES**

687 SI Figure 1. Piezometer Transect Across the Southwest Rim of Juniper Bay.

688 SI Figure 2. Piezometer Transect Across the Southeast Rim of Juniper Bay.

689 SI Figure 3. Piezometer Transect Across the Northeast Rim of Juniper Bay.

690 SI Figure 4. Piezometer Transect Across the Northwest Rim of Juniper Bay.

691 SI Figure 5. The apparatus used for collection of atmospheric deposition of P, alongside a tipping
692 bucket rain gauge and a traditional rain gauge. The sample collection bottle is not shown.