

1           **WATER QUALITY AND PLANKTONIC MICROBIAL ASSEMBLAGES OF**  
2                   **ISOLATED WETLANDS IN AN AGRICULTURAL LANDSCAPE**

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16 **Abstract**

17 Wetlands provide ecosystem services including flood protection, water quality  
18 enhancement, food chain support, carbon sequestration, and support regional biodiversity.  
19 Wetlands occur in human-altered landscapes, and the ongoing ability of these wetlands to  
20 provide ecosystem services is lacking. Additionally, the apparent lack of connection of some  
21 wetlands, termed geographically isolated, to permanent waters has resulted in little regulatory  
22 recognition. We examined the influence of intensive agriculture on water quality and planktonic  
23 microbial assemblages of intermittently inundated wetlands. We sampled 10 reference and 10  
24 agriculturally altered wetlands in the Gulf Coastal Plain of Georgia. Water quality measures  
25 included pH, alkalinity, dissolved organic carbon, nutrients (nitrate, ammonium, and phosphate),  
26 and filterable solids (dry mass and ash-free dry mass). We measured abundance and relative size  
27 distribution of the planktonic microbial assemblage ( $< 45 \mu\text{m}$ ) using flow cytometry. Water  
28 quality in agricultural wetlands was characterized by elevated nutrients, pH, and suspended  
29 solids. Autotrophic microbial cells were largely absent from both wetland types. Heterotrophic  
30 microbial abundance was influenced by nutrients and suspended matter concentration.  
31 Agriculture caused changes in microbial assemblages forming the base of wetland food webs.  
32 Yet, these wetlands potentially support important ecological services in a highly altered  
33 landscape.

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36

37 **Introduction**

38 Intermittently inundated wetlands of the southeastern USA provide important ecosystem  
39 services and values (Golladay et al. 1997, Semlitsch and Brodie 1998, Battle and Golladay 1999,  
40 Kirkman et al. 1999); these wetlands have been shown to support speciose plant (Kirkman et al.  
41 2004, Kaeser and Kirkman 2009) and amphibian communities (Liner et al. 2008), and are  
42 important for movement and breeding of reptiles (Subalusky et al. 2009). Many of these  
43 wetlands, often referred to as geographically isolated (e.g. Martin 2010), occupy shallow basins  
44 entirely surrounded by upland land cover. Their hydrology is variable, but they tend to have  
45 ponded water during periods when rainfall exceeds evapotranspiration (Kirkman et al. 1999). In  
46 the USA, their apparent lack of connection to perennial waters has resulted in little recognition  
47 and protection compared to other wetlands under Section 404 of the Clean Water Act (National  
48 Research Council 1995, Federal Register 1996). Isolated wetlands enhance water quality (Knox  
49 et al. 2008, Brown et al. 2010) and support biologically rich communities (Kirkman et al. 2004,  
50 Liner et al. 2008), however the potential for these processes and communities to persist in  
51 agriculturally altered landscapes is largely unknown.

52 Isolated wetlands are easily drained and have been significantly altered by agriculture  
53 practices (e.g., channelization, center pivot irrigation, runoff, and agrochemical application)  
54 (Bennett and Nelson 1990, Moreno-Mateos 2008). Globally, substantial wetland areas have  
55 been lost due to drainage and development. Over 50% of the area of depressional wetlands,  
56 riparian zones, floodplains, peatlands, and lake littoral zones has been lost mostly due to land  
57 conversion into intensive agriculture in North America, Europe, and Australia (Millennium  
58 Ecosystem Assessment 2005). The impact of wetland drainage on water storage and nutrient  
59 retention has received a great deal of attention, but changes and associated structural alteration of

60 wetlands in developed landscapes has not been well-studied. The impact of structural changes  
61 and nutrient supply on wetlands can be large (Armentano 1980, McCarty and Ritchie 2002).  
62 Previous studies have indicated that primary production in unaltered wetlands is nutrient-limited  
63 (Watt and Golladay 1999, Craft and Casey 2000, Battle and Golladay, 2001), thus wetland  
64 functioning is likely altered by elevated nutrient inputs.

65 Bacteria are likely the most abundant organisms in wetlands (Boon 2006), and are  
66 important contributors to biogeochemical functions (nutrient cycling, decomposition,  
67 assimilation of dissolved organic carbon, etc.) in intermittent wetlands (Palmer et al. 1997, Boon  
68 2006). Planktonic bacteria are important in food webs because they are consumed by  
69 zooplankton and, in turn, other macroinvertebrates (Boon and Shiel 1990, Thouvenot et al.  
70 1999). However, the influence of wetland alteration on planktonic microbes has been  
71 understudied. We examined water quality and associated planktonic microbial community within  
72 reference and agricultural wetlands of the Gulf Coastal Plain of the southeastern USA to  
73 determine the impact of agriculture (notably, elevated nutrients) on the microbial community.  
74 Our goal was to examine whether intensive agriculture influenced the abundance of planktonic  
75 microheterotrophs and what water chemistry variables best predicted microbial abundance.

76

## 77 **Methods**

### 78 ***Study Sites:***

79 Our study was conducted in the Dougherty Plain physiographic district of the Coastal  
80 Plain of Georgia, USA. The wetlands we sampled are considered geographically isolated,  
81 meaning that they are surrounded by upland vegetation/land use and are not directly connected  
82 by surface drainage to streams, lakes, or other permanent water bodies (Kirkman et al. 1999).

83 The isolated wetlands of southwestern Georgia often occupy shallow catchments that extend  
84 beyond the jurisdictional wetland boundary (Watt and Golladay 1999). The climate in this region  
85 is humid subtropical (Christensen 1981), with an average annual precipitation of 131 cm that is  
86 distributed evenly throughout the year. Mean daily temperatures range from 21° to 34°C in  
87 summer and 5° to 17°C in winter (National Climate Data Center, Asheville, NC). The area  
88 contains extensive agriculture dominated by peanut, cotton, corn, and cattle production  
89 (Golladay et al. 2000). We sampled 10 wetlands impacted by agriculture on privately owned  
90 working farms (center pivot irrigation, row crops, and cattle) and 10 reference wetlands on  
91 Ichauway, a 119-km<sup>2</sup> ecological reserve and site of the J.W. Jones Ecological Research Center,  
92 Baker County, Georgia (Fig. 1). Ichauway Reserve is a remnant longleaf pine (*Pinus palustris*)  
93 forest that has been relatively undisturbed since the 1930s, and has been managed with low  
94 intensity, dormant season prescribed fires (frequency of 1 to 3 years) for several decades.

95 ***Field Collection:***

96 Wetlands (Table 1) were sampled three times during the hydroperiod in 2009: in the  
97 winter before leaf-out (February), following a large rain event after leaf-out (April), and another  
98 leaf-out sampling period (June, during seasonal drying). In February, one of the sites could not  
99 be sampled because the wetland was dry (SA39.W2). In April, water over roads prevented us  
100 from sampling one site (SA39.W20). In June, we were able to sample all wetlands. Wetland  
101 sampling devices were constructed out of 5.1 cm diameter PVC pipe and placed in all the  
102 wetlands prior to the beginning of sampling so sample collection could be done with minimal  
103 disturbance to the water column. Devices were a vertical pipe embedded into wetland sediments  
104 until the pipe was stable and not subject to vibration during sampling (~ 30cm). A horizontal  
105 pipe was connected to the vertical pipe using a 90 degree connector. Small holes in the vertical

106 pipe above the sediment surface allowed free exchange of water with the water column. Clean,  
107 flexible tubing was inserted inside of the devices the day before sampling and then connected to  
108 an ISCO peristaltic pump the day of sampling. This enabled us to obtain water from the wetland  
109 water column without disturbing sediments. Samples for water chemistry and filterable solids  
110 (dry mass, DM and ash-free dry mass AFDM) were collected in 500 ml and 1000 ml acid  
111 washed and rinsed sample bottles. Three 10 ml samples for each wetland were collected during  
112 each sampling period for characterization of planktonic microbial assemblages via flow  
113 cytometry. Samples were placed on ice immediately following collection and kept refrigerated (4  
114  $\pm 1^\circ\text{C}$ ) until analysis.

115

116 ***Laboratory:***

117 *Water Chemistry –*

118 Samples were transported to the lab on ice and then filtered (Gelman A/E, GFF, 1-  $\mu\text{m}$   
119 nominal pore size). Dry mass (DM) and ash-free dry mass (AFDM) were determined  
120 gravimetrically (Wallace et al. 2006). Water chemistry was determined according to standard  
121 procedures (see Battle and Golladay, 2001b). We measured dissolved organic carbon (DOC) and  
122 dissolved inorganic carbon (IC) with a Shimadzu TOC-5050 analyzer (Shimadzu Scientific  
123 Instruments, Kyoto, Japan). We determined  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and soluble reactive phosphorus  
124 (SRP) with a Lachat Quikchem 8000 flow-injection colorimetric method (Lachat Instruments,  
125 Milwaukee, Wisconsin). Using unfiltered water, alkalinity and pH were assessed with a Mettler  
126 DL12 titrator (Mettler-Toledo Inc., Columbus, Ohio).

127 *Flow cytometry –*

128 Samples for flow cytometry were passed through a 45  $\mu\text{m}$  mesh sieve to remove large  
129 particles. Samples were preserved in formalin (2% final concentration) and kept at  $4\pm 1^\circ\text{C}$  in the  
130 dark (samples were analyzed within 1 month of collection). Samples were stained with the nucleic  
131 acid stain, SYBR Green II (SYBR, Invitrogen, final concentration 5X), for at least 30 minutes  
132 prior to analysis.

133 Flow cytometry was performed on a FACS Calibur flow cytometer (Becton Dickinson)  
134 using a 488 nm laser and a 635 nm red diode laser (for detection of chlorophyll  
135 autofluorescence). All parameters were logarithmically amplified and parameter values were  
136 displayed ranged four orders of magnitude on a log scale. Fluorescent beads (Calibrite, Becton  
137 Dickinson) were run periodically to verify that fluorescence intensity values remained consistent  
138 during sample analysis. SYBR Green II (SYBR) fluorescence was detected in a FL1  
139 photomultiplier tube (530/30 nm bandpass filter) and objects were first gated based upon FL1  
140 fluorescence ( $>50$  channels). Several samples were filtered (0.22  $\mu\text{m}$ ) and analyzed to verify that  
141 particles smaller than this size (e.g. colloids, viruses) were not detected using this threshold  
142 fluorescence value. Objects meeting the minimal FL1 threshold value were then gated based  
143 upon forward (FS) and side (SS) angle light scattering ( $>2$  channels).

144 To determine the sample volume analyzed, we measured the sample weight lost during  
145 analysis. This method yields accurate measurements of sample volume by adding a known  
146 quantity of beads to the sample (Rose et al. 2004). Between 70 and 100  $\mu\text{l}$  of sample was  
147 analyzed. Generally, the flow rate was set to keep the count rate below 1000 objects per second.  
148 For one of the agricultural wetlands (Striplings), samples were diluted 1:20 to keep the count rate  
149 near this limit.

150 Data generated via flow cytometry were analyzed using MatLab (V7.7, The Mathworks).  
151 Cells were gated according to chlorophyll *a* fluorescence, detected in FL3 (Chl *a* positive cells  
152 were >425 fluorescent channel values). The threshold value was determined to be appropriate for  
153 discriminating between autotrophic and heterotrophic bacteria based upon comparative analysis  
154 of cyanobacterial and heterotrophic bacterial cultures. This threshold value was sufficiently low  
155 to detect cyanobacterial Chl *a* fluorescence without detecting SYBR labeled heterotrophs. Large  
156 phytoplankton (e.g., phytoflagellates) produce greater Chl *a* fluorescence signals, therefore, were  
157 also capable of detection using this threshold. Fluorescence overlap between SYBR green and  
158 Chl *a* fluorescence was determined to be minimal. Cells without detectable Chl *a* fluorescence  
159 signals were described as heterotrophs. Both Chl *a* positive and negative cells were further  
160 classified based upon FS signal, which is a proximal measurement of cell size. The size gates  
161 were set based upon the modal FS value of 1.0, 2.0, 4.0  $\mu\text{m}$  diameter beads. For example, objects  
162 with a FS signal between the modal signal  $\geq 1.0$  and  $< 2.0$   $\mu\text{m}$  beads were grouped into the 1–2  
163  $\mu\text{m}$  size class. This categorization provides an estimate of cell size, however, because difference  
164 in morphology between spherical beads and cells, this estimate should not be used to calculate  
165 biomass. The final cell concentration for each group was calculated by adjusting for the flow  
166 rate, dilution (when samples were diluted), and the addition of small volumes of SYBR and  
167 internal reference bead suspensions.

168

#### 169 *Statistical Analysis-*

170 Water chemistry data were compared and analyzed separately by sample date due to the  
171 large variations in rainfall over the course of the study (Fig. 2). Water chemistry data were  
172 analyzed using a Mann Whitney Rank Sum Test since the majority of the data violated the



173 assumptions of normality. Dunn's test was used for pairwise comparisons. Principal Components  
174 Analysis (PCA) was used to illustrate the data in multivariate space and to reduce the number of  
175 water chemistry variables. Pearson's correlation was used to discern the variables that were  
176 related to the PCA scores.

177 Surprisingly, there were few cells containing chlorophyll in the water column, and only  
178 the heterotroph data were used for our analyses. Cell concentration (rather than biomass) was  
179 used to avoid biases caused by converting concentration to biomass without a thorough  
180 microscopic analysis of the microbial community (e.g., determining cell morphology).  
181 Heterotroph concentration data (cells mL<sup>-1</sup>) were natural log-transformed to meet the normality  
182 and the homogeneity of variance assumptions implicit in parametric analysis. Two-way  
183 ANOVAs were conducted to examine variation in number of small heterotrophs with wetland  
184 type and sampling period being the main effects. Tukey's Honest Significant multiple  
185 comparisons ( $\alpha = 0.05$ ; Littell et al. 2002) followed significant ANOVAs. However, this analysis  
186 does not indicate the strength of environmental factors influencing heterotrophy abundance. We  
187 were also interested developing predictor variables for the number of heterotrophs in a wetland.  
188 Water chemistry parameters differentiating reference and agricultural wetlands in the PCA were  
189 used in Akaike's Information Criterion (AIC) to determine the best linear model predicting the  
190 number of heterotrophs in the wetlands. We used pH, DM, AFDM, dissolved inorganic carbon  
191 (IC), NO<sub>3</sub>, PO<sub>4</sub>, and total dissolved carbon in the model building process. Several multiple linear  
192 models were calculated and compared using AIC. Based on maximum-likelihood estimates and  
193 the number of model parameters, AIC provides a measure for selecting among competing models  
194 of a given data set. The model having the lowest AIC is considered the best model because it  
195 provides the optimal compromise between predictive power and model complexity (see Johnson

196 and Omland 2004). AIC analysis allowed for determination of the water chemistry variables that  
197 likely lead to higher numbers of heterotrophic bacteria. An AIC was run for each individual  
198 sampling date to account for temporal differences. Then, an AIC was performed with all  
199 sampling dates with the cumulative rainfall 30 days preceding sampling added as a variable to  
200 the model. All analyses were done in SAS v9.2 (SAS Institute, Cary, NC).

201

## 202 **Results**

### 203 *Water chemistry-*

204 Water chemistry varied significantly between reference and agricultural wetlands (Fig.  
205 3). Suspended solids ( $p < 0.001$ ), pH ( $p < 0.001$ ), alkalinity (not shown;  $p < 0.001$ ), and soluble  
206 reactive phosphorus ( $p = 0.002$ ) were significantly higher in agricultural wetlands. Dissolved  
207 organic carbon was significantly different ( $p < 0.01$ , main effects model) and more variable in  
208 agriculturally impacted wetlands, however multiple comparisons were not able to distinguish  
209 differences among wetlands.

210 The PCA showed a strong grouping pattern distinguishing reference and agriculturally  
211 disturbed wetlands (Fig. 4). PC1 axes for all dates were generally related to higher pH, total  
212 suspended solids, nitrate, and phosphate. PC2 axes for all dates were related to higher dissolved  
213 carbon. Reference wetlands tended to show lower dispersion along both axes on all dates in  
214 comparison to agricultural wetlands.

215

### 216 *Heterotroph abundance-*

217 Heterotrophic cells (i.e., cells without chlorophyll fluorescence) dominated the water  
218 column in all wetlands. The majority of cells were small ( $< 4 \mu\text{m}$ ) and were likely bacteria

219 rather than heterotrophic eukaryotes. However, because the heterotrophic assemblage structure  
220 was not directly measured via microscopy, we used the term heterotrophic cells to describe this  
221 portion of the microbial assemblage. Yet, most are likely bacteria, with the larger cells consisting  
222 of flagellates. Greater than 94% of the heterotrophic assemblage was  $<4 \mu\text{m}$  at all sites, and  
223 larger cells became more abundant later in the season when the temperature increased; particles  
224  $< 2 \mu\text{m}$  composed 93% of the heterotroph assemblage in February (Fig. 5a), 80% in April (Fig.  
225 5b), and 84% in June (Fig. 5c). The total number of heterotrophs varied significantly with  
226 wetland type (agricultural vs. reference) and sampling period (Fig. 5; ANOVA,  $p < 0.0001$ ).  
227 Tukey's HSD indicated that heterotrophic bacterial abundance was greater in agricultural  
228 wetlands and that the April sampling date (Fig. 5b) was significantly different (i.e. lowest values  
229 in February (Fig. 5a) and highest in June (Fig. 5c)) in the number of heterotrophs than the other  
230 two sampling dates.

231 For the February sampling period, a model with DM, AFDM,  $\text{NO}_3$ , and  $\text{PO}_4$  best  
232 predicted the number of heterotrophs in the wetlands ( $w_m = 0.214$ ,  $R^2 = 0.98$ ; Table 2), with the  
233 next two models having a combination of those predictors. The April sampling followed a  
234 period of above-normal rainfall, which diluted the standing water (Fig. 2). This dilution affect  
235 increased the number of variables that distinguished the reference from the agricultural wetlands  
236 (Fig. 4) and increased the number of variables used in the AIC model selection. Heterotrophic  
237 bacterial concentrations sampled during April were best predicted by a model that included DM,  
238 AFDM, IC,  $\text{NO}_3$ , and  $\text{PO}_4$  ( $w_m = 0.452$ ,  $R^2 = 0.923$ ). The heterotroph assemblage present in June  
239 was best characterized by the amount of DM ( $w_m = 0.139$ ,  $R^2 = 0.85$ ). When sampling periods  
240 were combined into one model and an estimate for the 30 day total rainfall was added, a model  
241 that included DM, AFDM, IC,  $\text{NO}_3$ , and the total amount of rain over the previous 30 days best

242 predicted the heterotroph concentration ( $w_m = 0.255$ ,  $R^2 = 0.839$ ). Overall, the concentration of  
243 suspended matter and nutrients were drivers of heterotrophy cell concentration in these wetlands.

244

## 245 **Discussion**

246 Our study identified several key water chemistry variables that differentiated reference  
247 wetlands from agricultural wetlands (pH, suspended matter, nutrients, and dissolved carbon).  
248 Nutrient concentrations were generally elevated and showed a greater range of variability in  
249 agricultural relative to reference wetlands. Elevated nutrient levels are an indicator of fertilizer  
250 and animal waste runoff (Dorioz and Ferhi 1994, Carpenter 1998, Anctil et al. 2009). In addition,  
251 disturbed wetlands had higher pH and total alkalinity compared to reference sites, which we  
252 attribute to application of agricultural lime ( $\text{CaCO}_3$ ), a common practice on fields in  
253 southwestern Georgia (*personal observation*). Alkalinity and pH levels could also be influenced  
254 by irrigation, another common practice in the region. A majority of water used in irrigation  
255 comes from the upper Floridan Aquifer, which has higher pH and alkalinity than rainwater  
256 (Hicks et al. 1987). Cumulatively, these data suggest that agricultural areas in southwestern  
257 Georgia are contributing non-point source pollutants to adjacent isolated wetlands causing major  
258 changes in water quality.

259 Agricultural (row crop and pasture) wetlands showed further evidence of water quality  
260 alteration by having increased suspended sediment concentration. Sediment particles can have  
261 absorbed pollutants attached (Knox et al. 2008) and are a common non-point source pollutant  
262 associated with soil erosion from land development (Gaynor and Findlay 1995, Anctil et al.  
263 2009, Makarewicz et al. 2009). Agricultural wetlands had greater suspended particle

264 concentration than reference sites, which is evidence of increased runoff within and adjacent to  
265 the wetland boundary.

266         The concentration of heterotrophic microbial cells was generally greater in agricultural  
267 wetlands than reference sites. The planktonic heterotroph assemblage was dominated by  
268 organisms in the bacterial size range (i.e., < 4µm in cell size). In isolated wetlands, bacteria play  
269 key roles in major biogeochemical functions (nutrient cycling, decomposition, assimilation of  
270 dissolved organic carbon, etc.) (Boon 2006). Bacterial concentrations were an order of  
271 magnitude lower than found in previous studies of wetlands (Boon 1991). However, our study  
272 only investigated water column heterotrophs and did not include sediment bacteria. Due to these  
273 higher numbers of heterotrophs in the bacterial size range, water column process rates (e.g.,  
274 microbial uptake of nutrients, microbial transformations to gaseous forms, etc.) within these  
275 wetlands may be high and contribute substantially to ecosystem function.

276         This study identified several factors (suspended matter, nutrients, and over a longer time  
277 scale, cumulative rainfall) that influenced planktonic heterotrophic cell abundance. Many of  
278 these variables differentiated reference wetlands and agricultural wetlands (PCA; suspended  
279 sediments and nutrients), yet some variables did not seem to influence the abundance of  
280 heterotrophs (pH). In a previous study within the region, soil analysis indicated P or N/P co-  
281 limitation of isolated wetlands of southwestern Georgia (Craft and Casey 2000). Our results  
282 indicated that nutrients were important variables influencing planktonic heterotrophy abundance.  
283 We suggest that the timing of nutrient subsidies from agricultural fields, along with suspended  
284 sediments and rainfall are important drivers of planktonic heterotrophs in Coastal Plain isolated  
285 wetlands.

286 Numerous studies have demonstrated the importance of freshwater wetlands for  
287 maintaining water quality through remediation of nonpoint runoff (Johnston 1991, Mitsch and  
288 Gosselink 1993, Craft 1997, Knox et al. 2008). The importance of riparian and floodplain  
289 wetlands in sequestering N and P derived from fertilizer has been well documented (Brinson et  
290 al. 1981a, b, Naiman and Decamps 1997). Yet, biogeochemical functions of isolated wetlands  
291 are not well quantified. Isolated wetlands often occupy depressions in the landscape where they  
292 serve as focal areas concentrating non-point source runoff (Leibowitz 2003). Isolated wetlands  
293 assimilate nutrients associated with runoff through uptake in plant biomass and subsequent  
294 deposition in sediments, or through microbial denitrification in wetland soils (Whigham and  
295 Jordan 2003). While wetland soils are typically chemically reduced and contain ample organic C  
296 for denitrification (Craft 1997), this may not be the case in agricultural settings in which soil  
297 tillage and drainage aerate soils and promote loss of soil organic matter. Such practices are also  
298 likely to alter the chemical quality of organic carbon, which may alter rates of key microbial  
299 processes including denitrification. While it is well established that planktonic wetland  
300 communities (which here includes microbes, plants, and animals) can assimilate nutrients, their  
301 potential contribution to improving water quality of non-point source runoff to isolated wetlands  
302 remains poorly understood. Agricultural land management often results in reduction or  
303 elimination of rooted perennial vegetation, thus uptake by planktonic organisms may be a major  
304 pathway of nutrient assimilation and water purification. Our results show a clear response of  
305 planktonic microorganisms to non-point source runoff associated with intensive agriculture.

306 Agricultural land use has caused significant changes in the water chemistry and the  
307 associated heterotroph assemblage of isolated wetlands. Agricultural wetlands tended to have  
308 higher concentrations of planktonic bacteria, which seemed to be linked to availability of carbon

309 and nutrients. However, additional studies are needed to understand the impacts of other water  
310 quality variables (e.g., pH) on wetland planktonic communities. Further studies into nutrient  
311 processing and assimilation by wetland planktonic microbial communities will lead to greater  
312 insight on their potential for nutrient removal by isolated wetlands. Wetlands are known for their  
313 environmental remediation properties (Knox et al. 2008, Brown et al. 2010), however the  
314 ecosystem services (e.g., nutrient retention and cycling, water quality improvement) provided by  
315 isolated wetlands within altered landscapes are not well quantified. Excess fertilizer and manure  
316 on agricultural lands create surplus N and P, which is mobile in many soils and often leaches to  
317 downstream aquatic ecosystems and groundwater (Carpenter 1998). However, wetlands that  
318 remain in agricultural areas potentially trap nutrients might otherwise runoff into adjacent  
319 streams or into the groundwater (Knox et al. 2008). Our study indicates greater planktonic cell  
320 numbers in wetlands influenced by agriculture, which may be crucial to assimilation and cycling  
321 of nutrients (Knox et al. 2008). Thus, isolated wetlands potentially provide valuable ecosystem  
322 services within highly disturbed agricultural landscapes, although their potential remains largely  
323 unrecognized.

324

325

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Table 1: The size and the surrounding land use of the wetlands used in this study. Land use is delineated by a 100-m buffer surrounding each of the wetlands. Reference wetlands are surrounded by a fire-maintained second-growth longleaf pine (*Pinus palustris*) forest.

		<b>Wetland Area</b>	<b>%</b>		<b>% Row</b>	<b>%</b>
<b>Wetland</b>		<b>(m<sup>2</sup>)</b>	<b>Agriculture*</b>	<b>% Pasture**</b>	<b>Crop</b>	<b>Other***</b>
Agricultural	Westside	12900	45	94	0	55
	Eastside	15300	49	88	0	51
	W12	4400	22	78	0	88
	W16	22600	100	36	64	0
	W20	73800	49	0	49	51
	W2	22700	51	0	51	49
	W6	20500	98	0	98	2
	W5	24800	86	0	86	14
	Striplings	2900	74	74	0	26
	Skanky	37500	31	0	31	69
Reference	P2	60400	0	0	0	0
	P3	46900	0	0	0	0
	P4	172000	0	0	0	0
	P15	254000	0	0	0	0
	P21	84700	0	0	0	0
	P42	25900	0	0	0	0
	P46	113000	0	0	0	0
	P52	69500	0	0	0	0

P53	47500	0	0	0	0
P58	81800	0	0	0	0

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\* all agricultural activities; includes row crop and pasture

\*\* includes improved and unimproved pasture

\*\*\* includes silviculture development, and unimproved woodlands

Table 2: Results from the AIC model selection. The best three models from each sampling period and all dates combined are shown.  $K$  describes the number of variables in the model. The  $\Delta i$  is the difference between the AIC of the best fitting model and that of model  $i$ . The  $w_m$  is the normalized relative likelihood values known as the model weights.

Date	Parameters in Model	K	F-value	R <sup>2</sup>	AIC	$\Delta i$	$w_m$
Feb.	DM, AFDM, NO <sub>3</sub> , PO <sub>4</sub>	4	156.6	0.980	410.9	0.000	0.214
2009	DM, AFDM, PO <sub>4</sub>	3	196.4	0.977	411.3	0.380	0.177
	DM, NO <sub>3</sub> , PO <sub>4</sub>	3	183.4	0.975	412.5	1.583	0.097
April	DM, AFDM, IC, NO <sub>3</sub> , PO <sub>4</sub>	5	31.0	0.923	492.8	0.000	0.452
2009	pH, DM, AFDM, IC, NO <sub>3</sub> , PO <sub>4</sub>	6	24.3	0.924	494.5	1.682	0.195
	AFDM, IC, PO <sub>4</sub>	3	36.6	0.880	497.2	4.393	0.050
June	DM	1	79.4	0.850	417.0	0.000	0.139
2009	DM, IC	2	39.4	0.858	418.1	1.095	0.080
	DM, NO <sub>3</sub>	3	38.5	0.856	418.4	1.426	0.068
All	DM, AFDM, IC, NO <sub>3</sub> , Rain	5	48.8	0.839	1372.6	0.000	0.255
dates	DM, AFDM, IC, NO <sub>3</sub>	4	57.0	0.826	1374.5	1.908	0.098
	pH, DM, AFDM, IC, NO <sub>3</sub> , Rain	6	39.9	0.839	1374.6	1.953	0.096



## Figure Legends

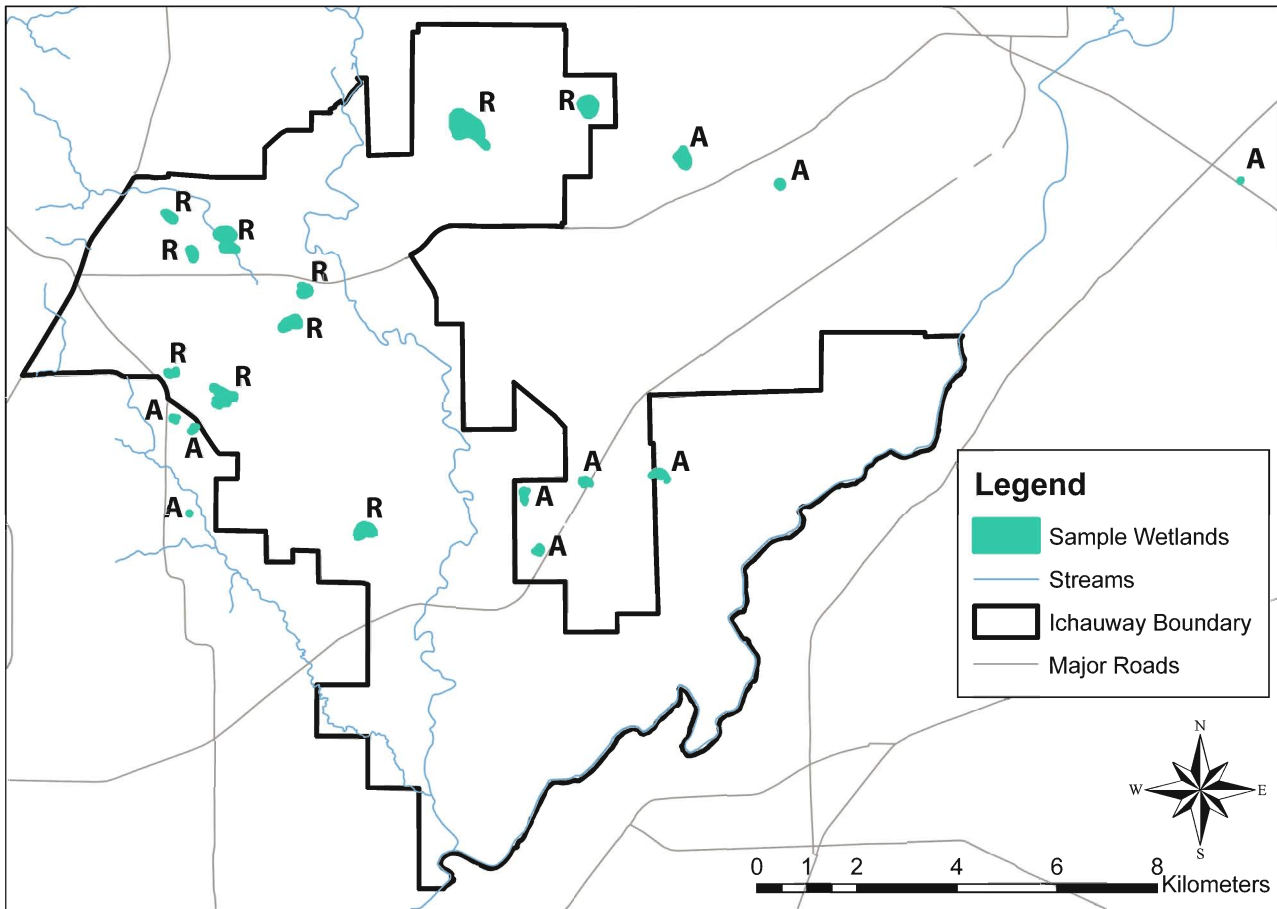
Fig 1 Sample sites were located within and near Ichauway. The sites (R - reference; A - agricultural) used in this study are labeled.

Fig 2 Daily rainfall totals for the sampling area. Arrows depict the sampling periods (February 2009, April 2009, and June 2009).

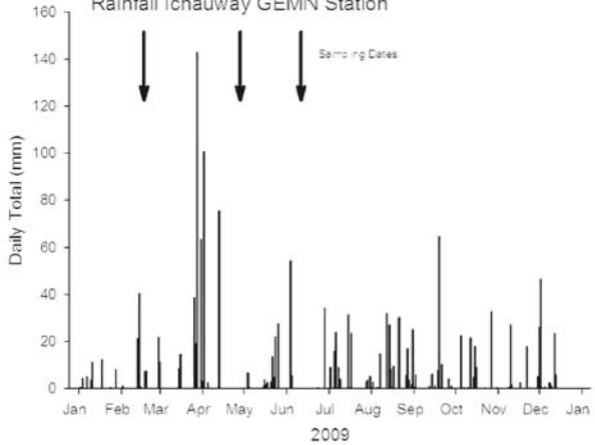
Fig 3 Averaged water chemistry results for agricultural and reference wetlands. Asterisks denote significant differences.

Fig 4 Results from the PCA illustrating how reference and agricultural wetlands group out in multivariate space. Arrow along the bottom of the axes indicate the variables that were important for the axis score.

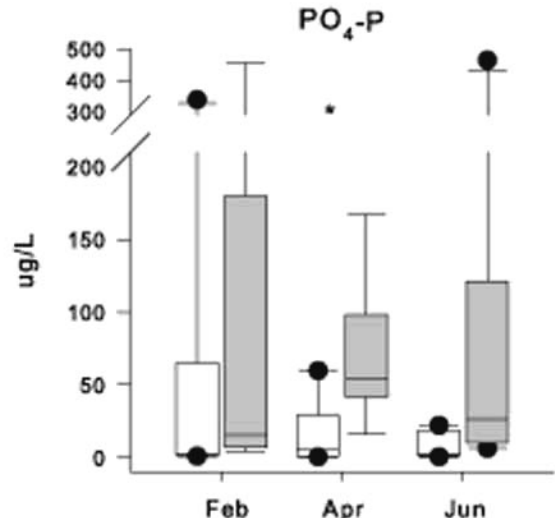
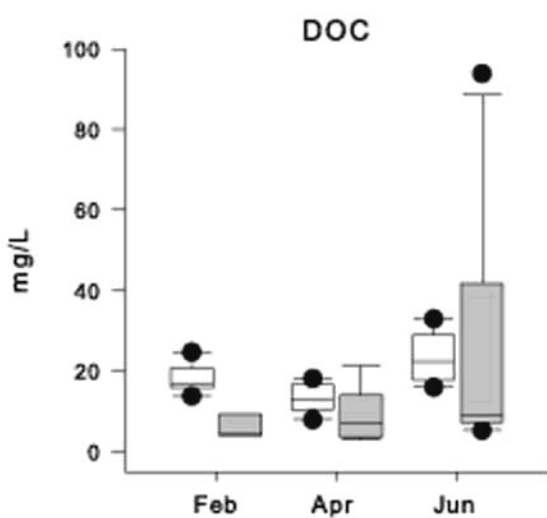
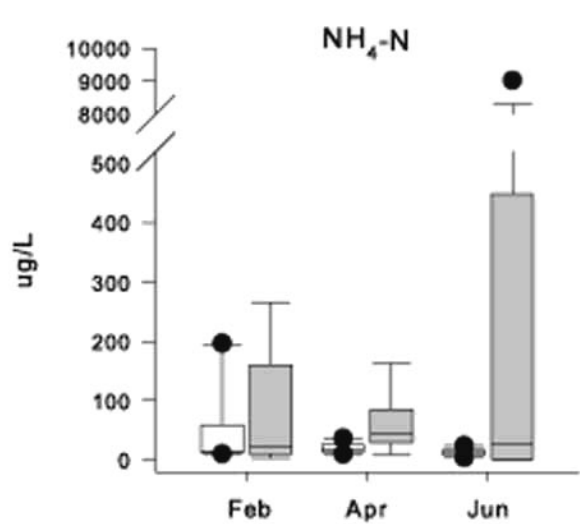
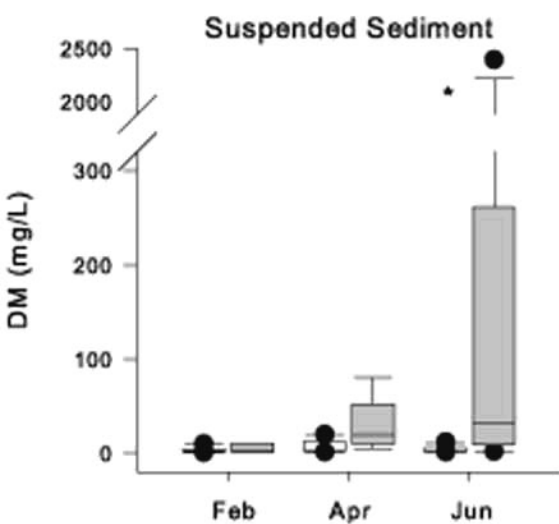
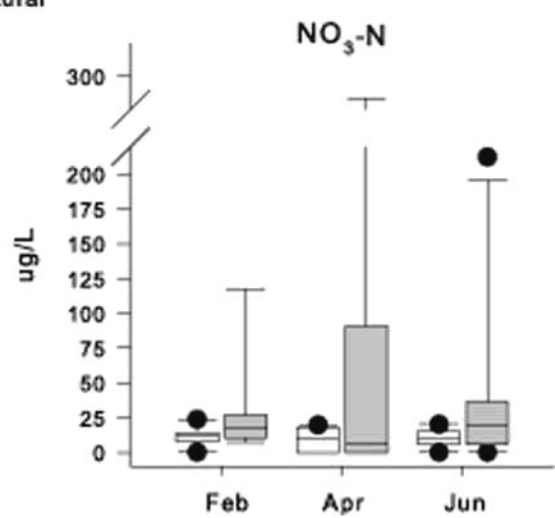
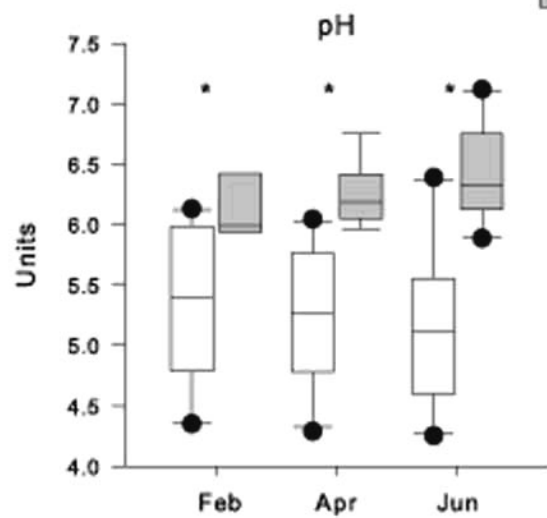
Fig 5 The total heterotroph concentration in all the ponds during the February (a), April (b), June (c) sampling periods, and the mean concentration ( $\pm$ SE) across all sampling periods (d). The total numbers of heterotrophs are categorized as size classes in A-C.



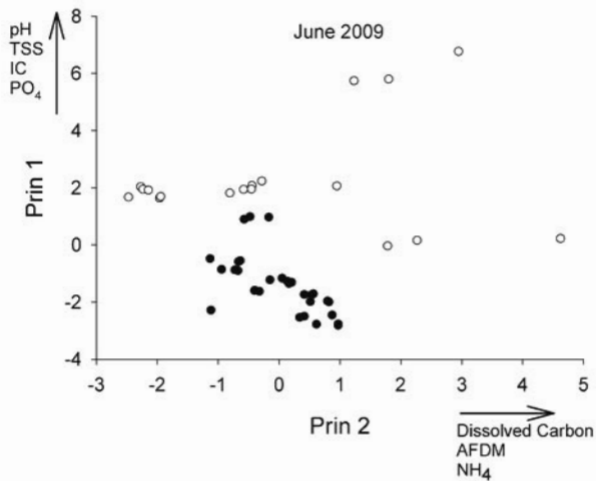
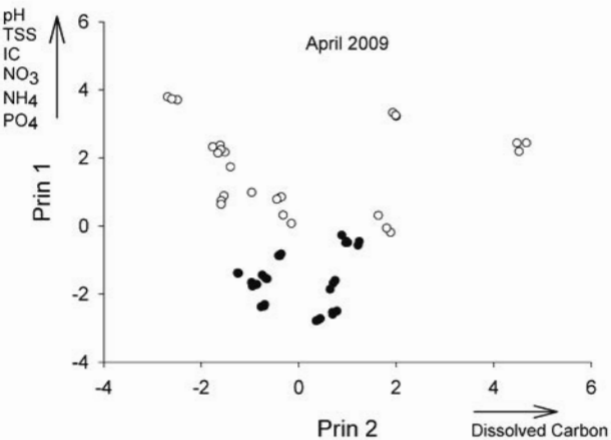
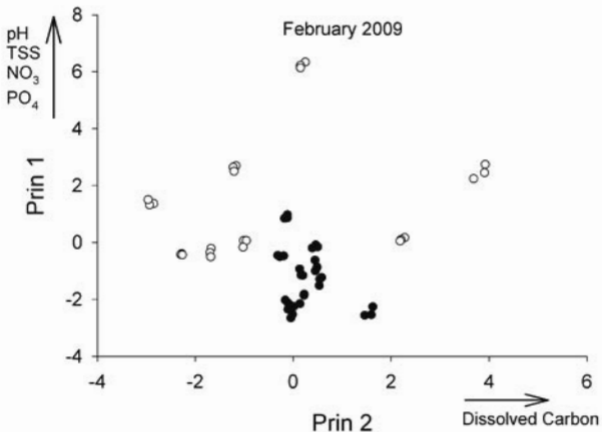
# Rainfall Ichauway GEMN Station



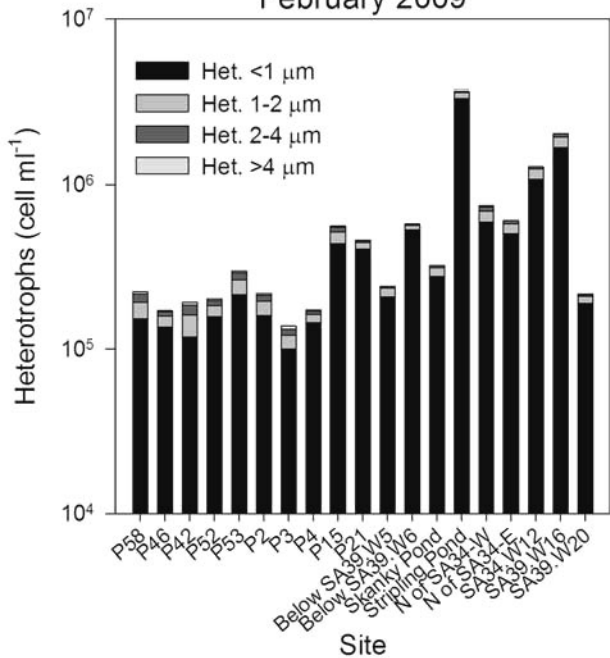
Reference  
Agricultural



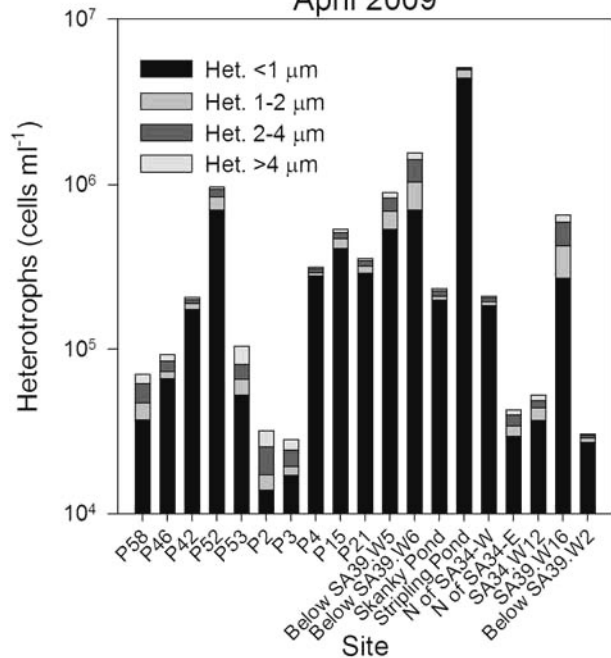
- Reference Wetlands
- Agricultural Wetlands



February 2009



April 2009



June 2009

