Impact of conservation land management practices on soil microbial function in an agricultural watershed

H.L. Tyler, M.A. Locke, M.T. Moore, and R.W. Steinriede

Abstract: The USDA Conservation Reserve Program (CRP) involves removing agricultural land from production and replanting with native vegetation for the purpose of reducing agriculture’s impact on the environment. In 2002, part of the Beasley Lake watershed in the Mississippi Delta was enrolled in CRP. In addition, areas between the lake and agricultural row crop (RC) fields were established as vegetative buffers (VB) to provide habitat for wildlife. Although the VB were established to improve wildlife habitat, an additional ecosystem service might include serving as an impediment to runoff from adjacent upland areas. The purpose of the current study was to assess the long-term impact of CRP, VB, and RC land management practices on the soil microbial community as an indicator of soil health. Soil samples were collected at two depths (0 to 5 and 5 to 15 cm [0 to 1.97 and 1.97 to 5.91 in]) from 12 sites within each land management (CRP, VB, and RC) area. Samples were assayed for soil enzyme activities (phosphatase, β-glucosidase, N-acetylglucosaminidase [NAGase], and fluorescein diacetate [FDA] hydrolysis) and microbial biomass. All enzyme activities were significantly higher in CRP and VB than in RC soils in the 0 to 5 cm depth. Microbial biomass in 0 to 5 cm soil was higher in CRP than in VB or RC areas. Significant correlations between microbial biomass carbon (C) and the activities of phosphatase ($R^2 = 0.514; p < 0.0001$), glucosidase ($R^2 = 0.434; p < 0.0001$), and FDA ($R^2 = 0.371; p < 0.0001$) were observed, indicating higher extracellular enzyme activities noted in CRP and VB relative to RC soil may be partially due to a larger soil microbial community, although other factors, such as substrate availability, also appear to play a role. The greater size and activity of microbial communities in CRP and VB indicate they are better equipped to process excess nutrients and pesticides and may be a contributing factor to the effectiveness of these conservation practices in reducing the impact of agricultural runoff on downstream bodies of water.

Key words: Conservation Reserve Program—microbial biomass—soil enzyme activity—vegetative buffers

Production of agricultural crops results in the application of pesticides and fertilizers that can be transported in runoff from fields to rivers and downstream bodies of water. Agrochemicals can have negative impacts on these downstream ecosystems, from eutrophication due to excess nutrients (Tilman 1999) to toxicity and development effects on aquatic life from pesticide exposure (Graymore et al. 2001; Chandler et al. 2004). Much attention has been given to reducing the impact of agricultural production on the environment, and a number of management practices have been suggested for reducing the amount of pollutants in agricultural runoff (Locke et al. 2010). The Conservation Reserve Program (CRP) was established by the US Food Security Act of 1985 to protect erodible agricultural land from degradation. This program is administered by the USDA Farm Services Agency (USDA FSA) and involves removing land from crop production followed by replanting with native vegetation for the purpose of improving environmental quality. Implementation of CRP decreases runoff velocity and increases infiltration of runoff and retention of water in soils, thereby reducing the volume of runoff and amounts of sediment loss (Udawatta et al. 2006; Jiang et al. 2007; Cullum et al. 2010).

In 2004, USDA FSA established the conservation practice “Habitat Buffers for Upland Birds” (CP-33) under the Continuous CRP to provide habitat for upland birds by establishing native grass buffers along field margins. These buffers offer many of the same benefits of the CRP (Dabney et al. 2006). Vegetative buffers (VB) increase the infiltration of runoff in soil and decrease its velocity (Robinson et al. 1996). As a result, pollutants carried in runoff (suspended sediments, excess nutrients, and pesticides) are deposited in buffer soils, thereby acting as a filter for contaminants in runoff before exiting the watershed. Numerous studies have demonstrated the effectiveness of VB strips in decreasing the concentrations of nitrogen (N) species (Patty et al. 1997; Mendez et al. 1999; Lee et al. 2003); phosphorus (P) (Patty et al. 1997; Lee et al. 2003; Borin et al. 2004); and various pesticides, including atrazine (Patty et al. 1997; Arora et al. 2003), chlorpyrifos (Arora et al. 2003), dichlorprop-p (Klöppel et al. 1997), diflufenican (Patty et al. 1997), isoproturon (Klöppel et al. 1997; Patty et al. 1997), and metolachlor (Arora et al. 2003), in agricultural runoff.

An important factor to consider regarding management practices such as CRP and VB strips is the fate of agrochemicals once they have been deposited in soils (Staddon et al. 2001). Whether pollutants are sorbed to soil particles, taken up by plants, or broken down by microorganisms will impact the long-term efficacy of these land management practices at reducing downstream contamination. In the case of sorption to soil particles, more labile pollutants with weak to moderate soil sorption coefficients have lower retention rates in buffer soils and may be released downstream over time after successive runoff events (Arora et al. 2010). When taken up by plants, senescence of plant tissue may release these pollutants back into the environment (Kao et al. 2003; Kröger et al. 2007). However, when soil microor-
ganisms act on agrochemical pollutants, the risk of them being reintroduced into agricultural runoff and carried downstream is minimized since microbes can degrade pesticides (Zablotowicz et al. 1998) and convert N to gaseous forms through processes such as denitrification (Groschen et al. 1991). In addition, extracellular enzyme production by an active microbial community promotes decomposition of plant litter and nutrient cycling in soils (Sinsabaugh and Moorhead 1994; Burns et al. 2013).

The Beasley Lake watershed in Sunflower County, Mississippi, drains an area of approximately 625 ha (1,544 ac). This watershed was maintained under row crop (RC) production (primarily cotton [Gossypium hirsutum L.] and soybean [Glycine max. L.]). In the spring of 2003, 113 ha (279 ac) was converted to CRP by planting with eastern cottonwood (Populus deltoids), oak (Quercus sp.), and hickory (Carya sp.). In 2006, 4 to 5 ha (10 to 12 ac) areas adjacent to agricultural RC fields on the southern side of the lake were established as VB to provide habitat for wildlife (Locke et al. 2008). Although the vegetative buffers were established to improve wildlife habitat, an additional ecosystem service might include serving as an impediment to runoff from adjacent upland areas. Following implementation of these and other management practices, improvements in the water quality of Beasley Lake were observed (Locke et al. 2008). Therefore, the purpose of the current study was to assess the long-term impact of these land management practices on the soil microbial communities in the Beasley Lake watershed as an indicator of soil health, looking at parameters linked to ecosystem service and remediation potential for pollutants commonly found in agricultural runoff.

Materials and Methods

Sampling Sites and Sample Collection.

Twelve sites were selected under each land management practice in the Beasley Lake watershed: Conservation Reserve Program (CRP), VB strips, and RC fields that had been planted in soybean the previous year (figure 1). Sampling sites within each land management practice were selected to equally represent silty loam and clay soil textures using previous soil surveys of the watershed (Locke et al. 2008; Soil Survey Staff 2012). Within each land management area, soil samples were collected from the following soil classifications: six sites from fine-silty, mixed, thermic Typic Endoaquolls; three sites from very-fine, smectitic, thermic Histic Dystrualfs; and three sites from very-fine, smectitic, thermic Vertic Epiaquolls. In April of 2012, soil samples were collected at two depths (0 to 5 and 5 to 15 cm [0 to 1.97 and 1.97 to 5.91 in]) from each of the 36 sites using a 5 cm diameter soil probe. For each site, six replicate cores were combined and mixed to produce a composite sample for each site and depth. Soil samples were passed through a 0.8 mm (0.03 in) sieve and stored field moist at 4°C (39.2°F) until further processing and analysis.

Enzymatic Activity.

All samples were assayed for phosphatase, β-glucosidase, and N-acetylglucosaminidase (NAGase) in a 96-well plate format using para-nitrophenol (pNP)-linked assays as described previously (Jackson et al. 2013). Fluorescein diacetate (FDA) hydrolysis was assayed using a protocol modified from Schnirer and Rosswall (1982). Briefly, 4 g (0.14 oz) of soil was transferred into 50 mL (1.69 oz) polypropylene centrifuge tubes and 15 mL (0.5 oz) of 50 mM sodium phosphate buffer (pH 7.6) was added. Triplicate tubes from each sample were treated with 250 µL of FDA stock solution (2 mg mL⁻¹ in acetone), vortexed, and incubated at 30°C (86°F) for one hour with shaking. Non-FDA controls were included for each sample. Reactions were stopped by the addition of 15 mL acetone, shaken for an additional 3 to 5 minutes, and centrifuged at 8,000 rpm for 10 minutes. Absorbance of supernatants was measured at 490 nm, and concentration was calculated by comparison to a standard curve.

Microbial Biomass and Soil Organic Matter.

Microbial biomass was determined using the chloroform fumigation extraction method (Horwath and Paul 1994). Briefly, 24 g (0.84 oz) fresh weight of chloroform fumigated and unfumigated subsamples from each site and depth were extracted with 100 mL (3.4 oz) of 0.5 M potassium sulfate (K₂SO₄) for one hour with shaking. Extracts were gravity filtered through #1 Whatman
filter paper (GE Healthcare, Pittsburgh, Pennsylvania), then divided into two 50 mL (1.69 oz) aliquots, one for determination of total C on an Apollo 9000 Combination TOC Analyzer (Teledyne Tekmar, Mason, Ohio) and another for determination of Total Kjeldahl N as described by Moore et al. (2010). Microbial biomass C (MBC) and microbial biomass N (MBN) were calculated using the following equations:

\[ \text{MBC} = E_C \times k_{EC} \text{ and } \]
\[ \text{MBN} = E_N \times k_{EN} \]

where \( E_C \) and \( E_N \) are the difference in C and N between chloroform fumigated and unfumigated subsamples, and \( k_{EC} \) and \( k_{EN} \) are constants estimated at 0.35 and 0.68, respectively (Horwath and Paul 1994). Soil organic matter (SOM) was determined by ashing oven dried soils at 500°C (932°C) for two hours and reported as the percentage of dry matter burned off by ashing.

**Statistics.** Analyses on enzyme activities and microbial biomass were performed in JMP version 11.2.0. (SAS Institute Inc., Cary, North Carolina). Two-way analysis of variance (ANOVA) was conducted to determine effects of land management type (RC, VB, or CRP) and depth (0 to 5 and 5 to 15 cm [0 to 1.97 and 1.97 to 5.91 in]) on enzyme activities and microbial biomass. Correlations between enzyme activity and MBC, MBN, and SOM were determined by linear regression. All analyses were assessed at an \( \alpha \) of 0.05.

**Results and Discussion**

The CRP and VB land management practices are employed for the improvement of environmental quality and wildlife habitat. When land is taken out of RC production and put under CRP, microbial biomass and other biological indicators show greater and more rapid improvement than other soil quality parameters (Karlen et al. 1998, 1999). Therefore, the current study investigated soil microbial characteristics of RC fields as compared to land under CRP and VB, nine and six years, respectively, after conversion from agricultural production. Generally, both enzyme activities and MBC were higher and more variable at the 0 to 5 cm (0 to 1.97 in) depth in CRP, VB, and RC soils. These observations are in agreement with Follett et al. (2001), who reported effects of CRP are most evident in 0 to 5 cm surface soils. Purakayastha et al. (2009) also found 0 to 5 cm soils contained higher levels of MBC than deeper 5 to 10 cm (1.97 to 3.94 in) soils.

MBC differed among all three land management practices at the 0 to 5 cm depth (table 1), with levels in both VB and CRP being significantly higher than RC soils \( (p < 0.0001) \). MBC was also greater in CRP than in VB soils \( (p = 0.0008) \). Conversion of agricultural fields to CRP has been shown to significantly increase the levels of MBC in soils (Karlen et al. 1999; Acosta-Martínez et al. 2008; Purakayastha et al. 2009; Follett et al. 2015). Most of the these studies were conducted in the northwestern and central regions of the United States, including the Great Plains, Corn Belt, Northern Plains, and Columbia Plateau. The current study demonstrates this increase of MBC in CRP also occurs in Mississippi Delta soils where the average daily temperature is 15.5°C (59.9°F) and the average annual rainfall is 127 cm (50 in). The magnitude of this increase \( (<200\%) \) is also similar to other studies finding close to 200% and 175% more MBC in CRP relative to RC soils (Karlen et al. 1999; Acosta-Martinez et al. 2008). Purakayastha et al. (2009) found CRP land had only 40% more MBC than conventionally tillled land in surface 0 to 5 cm soils. The difference in the extent of MBC recovery in CRP lands between these studies may be due to differences in vegetation (brome grass in the Purakayastha et al. [2009] compared to hardwood trees in the current study). However, fewer studies have looked at the levels of MBC in VB soils, Reungsang et al. (2001) found MBC in a five-year-old switchgrass (Panicum virgatum L.) buffer was greater than an adjacent corn (Zea mays L.)–soybean field in the top 15 cm (5.91 in) of soil.

MBN was significantly higher in 0 to 5 cm (0 to 1.97 in) CRP soil than either VB or RC soil (table 1; \( p \leq 0.0003 \)). When compared to RC soils, MBN in CRP was 300% higher, which corresponds with other studies reporting the amount of MBN in CRP surface soils was more than triple the amount found under continuous cotton (Acosta-Martínez et al. 2008). Neither MBC nor MBN differed significantly between CRP, VB, or RC in the 5 to 15 cm (1.97 to 5.91 in) soils (table 1), which is in agreement with Purakayastha et al. (2009), who observed MBC in deeper soils was less impacted by land management type. Taken together, the higher levels of MBC and MBN in VB and CRP compared to row cropped land demonstrates that implementation of these conservation practices in the Beasley Lake watershed has increased the number of microbial cells in soil. Such an increase in the level of soil microorganisms is likely to enhance the potential for VB and CRP lands to mitigate agricultural pollutants deposited in these soils.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Soil moisture, soil organic matter (SOM), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) in 0 to 5 and 5 to 15 cm soils under row crop (RC), vegetative buffer (VB), and Conservation Reserve Program (CRP) land management practices.</th>
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<td>Depth (cm)</td>
<td>Land management</td>
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<td>---------</td>
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</tr>
<tr>
<td>0 to 5</td>
<td>RC</td>
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<td></td>
<td>VB</td>
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<td>CRP</td>
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<td>5 to 15</td>
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<td>CRP</td>
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Notes: Values presented are the mean ± standard error (\( n = 12 \)). Statistical differences were determined by two-way ANOVA and are denoted by different letters (\( \alpha = 0.05 \)).
tional activities and provide insight on the mitigation potential of these soils.

Phosphatase activity was highest in CRP soils at both depths ($p \leq 0.0007$), while VB was only greater than RC in the 0 to 5 cm (0 to 1.97 in) soils ($p < 0.0001$; figure 2a). Phosphatase activity in soils is regulated by inorganic P availability, where there is an inverse relationship between phosphate (PO$_4^{3-}$) concentrations and phosphatase activity (Olander and Vitousek 2000; Nannipieri et al. 2011). Which suggests that the higher activity in CRP and VB compared to RC soils might be due to lower concentrations of inorganic P in these soils. Since phosphatase mineralizes organic PO$_4^{3-}$ forms from soil (Turner et al. 2002), higher activities observed in CRP and VB soils demonstrate they have a greater capacity for mineralizing organic PO$_4^{3-}$ to inorganic forms, making it more bioavailable for plant uptake.

$\beta$-glucosidase activity in both VB and CRP was significantly higher than in RC 0 to 5 cm (0 to 1.97 in) soils ($p < 0.0075$; figure 2b). In addition, the activity in CRP was higher than RC or VB soils at both depths analyzed ($p \leq 0.0191$). Glucosidase is a good overall indicator of soil quality as well as a soil’s ability to break down plant material (Stott et al. 2010). Thus, the higher $\beta$-glucosidase levels observed here indicate the quality of CRP and VB soils have improved relative to RC soils, and that VB and CRP soils have a greater ability to promote C cycling and processing of nutrients released from senescent plant tissues in these lands.

NAGase activity was also significantly higher in VB than RC surface 0 to 5 cm (0 to 1.97 in) soils ($p = 0.0002$), while activity in CRP was greater than RC at both depths ($p \leq 0.0001$; figure 2c). The RC field in the current study had been planted with soybeans and did not receive N fertilization. However, studies indicate 10% of soybean N can be released in root leachates during soybean growth, with the majority of this N in organic forms (Brophy and Heichel 1989).

Given that NAGase has been demonstrated to be a good index for N mineralization in soils (Ekenler and Tabatabai 2004; Tabatabai et al. 2010), the higher levels of this enzyme in VB and CRP soils indicate they are better equipped to process any organic N species deposited in soils by runoff.

In contrast to the activities of enzymes linked to nutrient mineralization, FDA hydrolysis in VB 0 to 5 cm (0 to 1.97 in) soils was significantly higher than in CRP soils ($p = 0.0024$), while both VB and CRP activities were greater than those observed in RC soils ($p \leq 0.0004$; figure 2d). FDA hydrolysis is considered a measure of overall hydrolytic activity, including the activities of esterases, lipases, and proteases from soil bacteria (Schnürer and Rosswall 1982). In addition, Zablotowicz et al. (2000) found FDA hydrolysis was strongly correlated with the esterification of the herbicide fenoxapropethyl, the first step in its breakdown. Therefore, higher FDA activities in VB and CRP indicate they may be better equipped to degrade fenoxapropethyl and related compounds. Given that plant root exudates can influence microbial communities in soil (Haichar et al. 2008), the higher FDA activities in VB and CRP are likely due to the presence of vegetation throughout the entire year, while RC fields lay fallow for six to seven months.

Similar to the results of the current study, the activities of $\beta$-glucosidase, glucosaminidase, and FDA hydrolysis were all higher in grassy buffers than in RC fields in an agricultural watershed in Missouri (Udawatta et al. 2009). In addition, a previous study conducted in the Beasley Lake watershed found phosphatase activity in VB strips were almost 1.9 times greater than an adjacent, unvegetated field, while FDA hydrolysis was 3.8 times higher (Staddon et al. 2001). Results of the current study also found VB phosphatase and FDA activities were greater than RC fields, but to a lesser extent, likely due to the difference in sampling depth (0 to 2 cm [0 to 0.79 in]) in Staddon et al. [2001] compared to 0 to 5 and 5 to 15 cm [0 to 1.97 and 1.97 to 5.91 in] in the current study.

In order to investigate if the size of the microbial community influenced enzyme activities in the Beasley watershed, linear regression analyses were conducted. These regressions revealed significant correlations between MBC and the activities of phosphatase ($R^2 = 0.514$; $p < 0.0001$; figure 3a), $\beta$-glucosidase ($R^2 = 0.434$; $p < 0.0001$; figure 3b), and FDA ($R^2 = 0.371$; $p < 0.0001$; figure 3d). Correlations between MBC and NAGase (figure 3c) and all enzyme activities and MBN were also significant (table 2), but to a lower extent, with $R^2$ values not exceeding 0.275. The lower $R^2$ value between MBC and NAGase appears to be driven by three samples from the 5 to 15 cm (1.97 to 5.91 in) CRP soils with high activity, but low microbial biomass compared to other samples (figure 3c). Five data points in the MBC versus FDA regression, all from 0 to 5 cm (0 to 1.97 in) VB soils (figure 3d), also fell outside of the linear trend observed. One potential explanation for these outliers is that the samples might contain a higher proportion of fumigation resistant members of the microbial community. For example, Ingham and Horton (1987) reported that chloroform fumigation only reduced fungal cells in soil by 9% to 59% and bacterial cells in soil by 50% to 99%. Therefore, the microbial community in these soils may have a higher proportion of fungi compared to other samples, or members of the bacterial community that are resistant to fumigation. This observation highlights the need for future studies examining microbial community composition in the Beasley Lake watershed.

While microbial biomass and enzyme activities can be correlated with each other, this is not always the case and the relationship can vary under different soils and environmental conditions (Nannipieri et al. 2002). Given the significant correlations of MBC with several of the enzyme activities investigated in the current study, it is likely the higher activities noted in CRP and VB relative to RC soil is partially due to a greater microbial biomass. However, since the $R^2$ values are not higher than approximately 0.5, other factors, such as substrate availability and SOM content (Sinsabaugh et al. 2008), are also likely to play a role in the higher enzyme activities observed in VB and CRP soils. Phosphatase, $\beta$-glucosidase, and NAGase activities had higher $R^2$ values when compared to SOM than MBC or MBN (table 2). Given that these enzymes are involved in nutrient cycling of organic matter, their significant correlations with SOM indicate substrate availability also contributes to their higher activities in VB and CRP soils.

Most studies examining vegetative buffers look at soil enzyme activities, but not microbial biomass, while studies examining the microbial communities in CRP soil measure microbial biomass, but not enzymes. To the authors’ knowledge, the current study is one of the few to report both microbial biomass and soil enzyme activities and compare them between CRP and buffer lands. In the current study, microbial biomass and enzyme activities (with the exception of FDA hydrolysis) in CRP land were higher than in VB (figure 2). One possible reason for the higher activities in CRP compared to VB is the greater length of time that CRP has been
Figure 2
Activities of (a) phosphatase, (b) β-glucosidase, (c) N-acetylglucosaminidase (NAGase), and (d) fluorescein diacetate (FDA) hydrolysis in soils from Conservation Reserve Program (CRP), vegetative buffer (VB), and row cropped (RC) lands reported as nmole of substrate consumed per hour per gram dry weight soil. Soils were collected from 0 to 5 cm (open bar) and 5 to 15 cm (closed bar) depths. Values represent mean ± standard error (n = 12). Statistically significant differences were determined by two-way ANOVA and are denoted by different letters.

Table 2
Statistics from linear regressions of phosphatase, β-glucosidase, N-acetylglucosaminidase (NAGase), and fluorescein diacetate (FDA) hydrolysis to microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and soil organic matter (SOM).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>MBC</th>
<th>MBN</th>
<th>SOM</th>
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<tr>
<td>β-glucosidase</td>
<td>0.434</td>
<td>0.245</td>
<td>0.517</td>
</tr>
<tr>
<td>NAGase</td>
<td>0.244</td>
<td>0.193</td>
<td>0.366</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>0.514</td>
<td>0.275</td>
<td>0.566</td>
</tr>
<tr>
<td>FDA hydrolysis</td>
<td>0.371</td>
<td>0.105</td>
<td>0.143</td>
</tr>
</tbody>
</table>

Notes: All values are R² statistics from linear regressions. All regressions were significant with p < 0.01.

established in the Beasley Lake watershed compared to the vegetated buffers. Research has demonstrated that the length of time a conservation practice has been in place can have a greater impact on soil quality than the type of practice. For example, Purakayastha et al. (2009) found crop land maintained under no till conditions for 28 years displayed greater MBC, total soil N, and N mineralization than land that had been under CRP for 11 years. Several other studies have noted that soil quality parameters measured in land under long-term CRP are still less than the level observed in native grasslands that have been undisturbed by agricultural production. Amelung et al. (2001) found
that after eight years under CRP, levels of SOM had only recovered by 20% compared to native grasslands. While land under CRP for ten years had significantly higher microbial biomass relative to soil from land newly enrolled in the program, this amount of time was not long enough for microbial biomass to recover to levels found in native prairies (Baer et al. 2000). In the Great Plains, a study spanning 14 different sampling sites found soil microbial biomass in agricultural land converted to CRP for 5 to 10 years was still not as high as native prairies (Follett et al. 2015). These results demonstrate that recovery of agricultural land back to native conditions after implementation of CRP is still a slow process.

Summary and Conclusions
Several studies have reported the impact of CRP on soil microbial characteristics. However, vegetation planted when establishing CRP will vary by region depending on native flora. To the authors’ knowledge, the current study is the first to look at microbial communities under long-term CRP in the Mississippi Delta region. Many papers reporting the microbiological quality and soil quality of CRP lands do so in comparison to cropped fields under different tillage and irrigations practices, while very few look at them in comparison to buffer strips. The current study provides a unique perspective comparing both CRP and VB soils to RC lands, as well as to each other, demonstrating these practices do help restore soil quality. With soils that possess a larger microbial community and higher enzyme activities linked to C, N, and P cycling (glucosidase,
NAGase, and phosphatase) and hydrolytic activity (FDA hydrolysis), lands maintained under these conservation practices are more capable of minimizing the impact of agricultural runoff on downstream ecosystems in the Mississippi Delta region.

References


