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NOTES AND CORRESPONDENCE

Factors Controlling Sediment Denitrification Rates in Grassland and Forest Streams

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ABSTRACT

Sediment denitrification is an important nitrate (NO_3^-) removal process from agricultural streams. The direct and indirect factors that control denitrification rates in tributary sediments can vary depending on the types of agricultural activities and vegetation. Our research investigated (1) tributary sediment denitrification rates in a grassland stream affected by pasture ecosystems and a forest stream affected by N fertilization; and (2) the environmental factors that determine denitrification rates in tributary sediments. The denitrification enzyme activity (DEA) in grassland stream sediments is positively correlated with precipitation likely due to the increased nutrient exchange rates between stream water and sediments, and was higher than in forest stream sediments, leading to a decrease in NO_3^- concentration ($[NO_3^-]$) in stream sediments. The DEA in riparian sediments was regulated by carbon concentrations and did not contribute to NO_3^- removal from the riparian sediment in grassland and forest streams. Thus, environmental factors affected by different types of agricultural activities and vegetation might regulate denitrification rates and $[NO_3^-]$ in agricultural stream ecosystems.

Key words: Denitrification enzyme activity, Santa Fe River Watershed, Tributary sediments

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1. INTRODUCTION

The Santa Fe River Watershed (SFRW) spreads across eight counties in North East Florida and comprises the southeastern part of the Suwannee River Basin that drains into the Gulf of Mexico. The SFRW comprises only 13% of the Suwannee River Basin area, but contributes 22% of the total N input to it, causing increased nitrate concentrations ($[NO_3^-]$) in spring, stream and ground waters (Suwannee River Water Management District 2003). Within the SFRW, agricultural land use including crop and improved pastures occupy over 37% of the land use. The agricultural area that is fertilized using N has been increasing (Sabesan 2004). High $[NO_3^-]$ leads to eutrophication (Burkart and James 1999) and loss of biodiversity (Galloway et al. 2004). Consumption of water with high $[NO_3^-]$ can cause blue baby syndrome (Ward et al. 1996). Consequently, N contamination in SFRW stream ecosystems due to agricultural N fertilization and manure from ranching is an increasing concern.

Microbial denitrification can remove up to 50 - 60% of incoming NO_3^- from agricultural streams (Green et al. 2004), but the process prefers anoxic conditions. Consequently the locations in which it occurs and its efficiency, differ among ecosystems. In streams, denitrification occurs primarily in sediments because denitrifying microbes attach to sediment particles (Duff and Triska 1990; García-Ruiz et al. 1998). In riparian sediments adjacent to streams denitrification removes NO_3^- from groundwater before it discharges into streams (Schipper et al. 1993) because these sediment are usually anaerobic due to water saturation and oxygen depletion due to decaying organic matter (OM).

Various direct and indirect environmental factors control sediment denitrification rates in agricultural ecosystems. Direct factors include the availability of NO_3^- and

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OM, dissolved oxygen concentrations and temperature (Birgand et al. 2007). Indirect factors include types of agricultural activities, vegetation, precipitation, soil texture and the location of the stream (Birgand et al. 2007). For example, grassland streams affected by pasture ecosystems receive organic C from manure and this C could supply additional electron donors to denitrifiers; while forest streams affected by N fertilization receive inorganic N (e.g., NH_4^+ and NO_3^-), which increases the supply of electron acceptors to denitrifiers. Furthermore, the slopes of streams can influence the nutrient residence time in the sediments, thereby affecting sediment denitrification rates. Thus, understanding the importance and role of the factors that indirectly affect denitrification rates is essential for N agricultural stream management. Our studies investigated how direct and indirect environmental factors affected denitrification rates in tributary sediments affected by different types of agricultural activities and vegetation. Specific objectives were (1) to compare the denitrification rates of grassland stream sediments affected by pasture ecosystems to those of forest stream sediments affected by N fertilization; and (2) to investigate the environmental factors that determine the rates of denitrification in the sediments of these streams.

2. MATERIALS AND METHODS

2.1 Site Description and Sampling

The study site included stream and riparian sediments from two tributaries (T1 and T2) at the Boston Farm Santa Fe Ranch Beef Unit Research Center (SFBRU) in the SFRW, Alachua County, FL, USA (Fig. 1a). Land uses on this site include a low-intensity cattle operation with about 300 heifers on 648 ha, and a tree nursery. T1 runs through a pasture ecosystem that is vegetated with grasses (*Juncusp*), forbs (*Saururus cernuus*, *Hydrocotle umbellate*, *Polygonump*) and trees (*Caryap*, *Pinus*, *Quercus*, *Magnolia grandiflora*, *Cephalanthus occidentalis*). T2 runs through a forest ecosystem vegetated with hardwoods (*Carya*, *Quercus*, and *Magnolia grandiflora*), softwoods (*Pinus*) and grass (*Juncus*) (Frisbee 2007). The upstream T2 region receives N loads (NH_4NO_3 and Urea) directly from a nursery operation. The average slope is lower at the T1 site (~0%) than at the T2 site (4.7%). The precipitation and temperature range during the sampling period (December 2006 - August 2007) were 3 - 20 cm and 18° - 29°C, respectively (US Geological Survey, National Elevation Dataset, downloaded from Florida Geographic Data Library). Four stream and riparian sediment samples

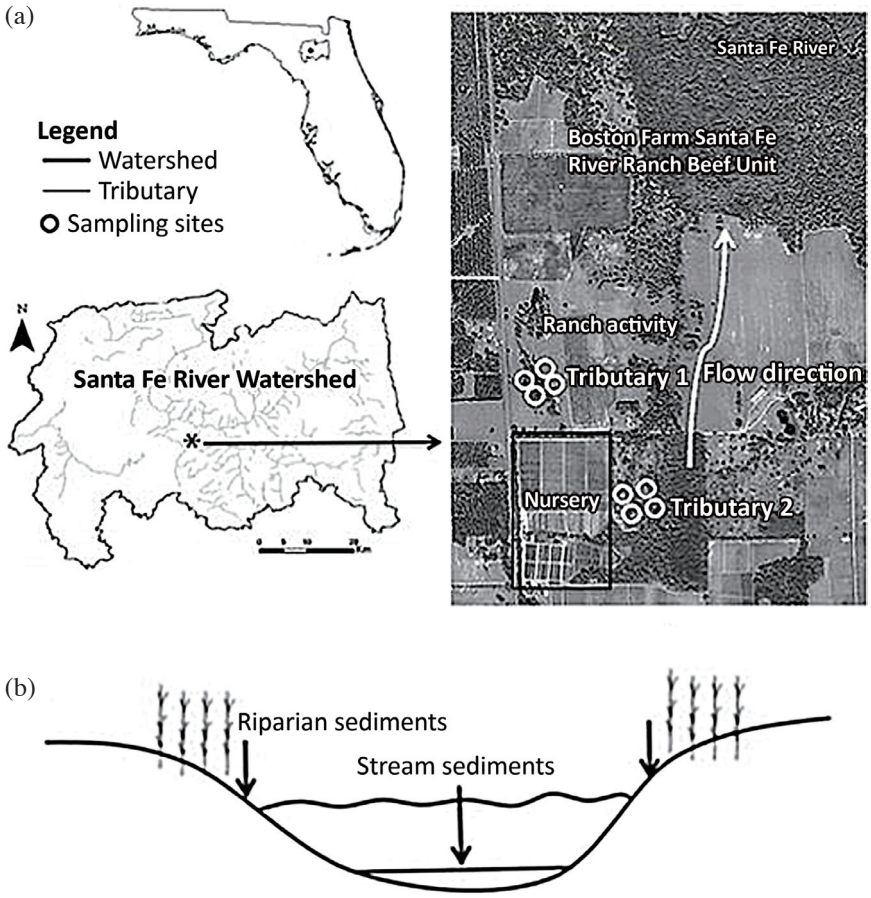

Fig. 1. Overview of sampling sites in the Boston Farm Santa Fe Ranch Beef Unit Research Center (a) and stream and riparian sediments (b) of the Santa Fe River Watershed, northern Alachua County, Florida.

were collected to a depth of 3 cm with a polyvinylate core (diameter 7.5 cm) from each of T1 and T2 for December 2006, March, May, July and August 2007 (Fig. 1b). Collected sediment samples were transported to the laboratory on ice and stored at 4°C until analysis. All roots and litter were removed from the sediment before analysis.

2.2 Analyses of Chemical Properties and Denitrification Enzyme Activity

The pH and dissolved oxygen concentrations in the stream water were determined *in situ* using YSI (Cole-Parmer, USA). Inorganic nitrogen (NH_4^+ and NO_3^-) was extracted from sediment samples using 0.5 M K_2SO_4 (Bundy and Meisinger 1994); the supernatant was filtered and analyzed. NH_4^+-N content was quantified using a Seal AQ2 automated Discrete Analyzer [Mequon, WI, USA; EPA Method 350.1 (O'Dell 1993a)]. NO_3^--N content was analyzed using an Alpkem Rapid Flow Analyzer 300 Series [Clackamas, OR, USA; EPA Method 353.2 (O'Dell 1993b)]. Total extractable organic carbon concentration (Ext. Org C) was measured using a Shimadzu TOC-5050A Total Organic C Analyzer equipped with an ASI-5000A auto sampler (Kyoto, Japan). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in the sediments were determined by the differences between the original samples, and samples treated using chloroform fumigation-extraction (Brookes et al. 1985).

Denitrification enzyme activities (DEA) were determined using an acetylene blocking method (Tiedje 1988). Ten grams of wet sediments were added into a 160-mL serum bottle, amended with 1400 μg of NO_3^--N (as KNO_3), 8 mL of 4 mM glucose solution, 2 mL of 0.5 M chloramphenicol and 20 mL of acetylene gas (12.5% of 160-mL serum bottle), then incubated for 4 h at 20°C. The amount of N_2O in the headspace was measured at pre-determined time intervals up to 4 h using a Shimadzu Gas Chromatograph 14-A equipped with a Nickel-63 electron capture detector (Ni-63 ECD; Kyoto, Japan). Operating temperature was 120°, 30°, and 230°C for the injection, column and detector respectively. Potential denitrification activity (PD) was measured using the same method above mentioned except for no glucose addition.

2.3 Statistical Analysis

Statistical analyses were conducted using JMP version 10.0 (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance tests (ANOVA) were performed to compare chemical properties and denitrification rates between sites. Least significant difference ($\alpha = 0.05$) was used for comparisons. Regression analysis was used to quantify relationships between denitrification rates and chemical properties.

3. RESULTS

In stream sediments, [NO_3^-] was significantly higher at the T2 site than at the T1 site; (Table 1; $p < 0.05$). In riparian sediments, [NO_3^-] were not significantly different between sites. In both sediments, DEA was higher at the T1 than at the T2 site (Table 1; $p < 0.05$). All tested chemical concentrations were higher in riparian sediments than stream sediments, but the differences were not significant (Table 1).

The DEA of stream sediments was the highest in July 2007 at the T1 site, and in July and August 2007 at the T2 site (Fig. 2a). At the T1 site, DEA was positively correlated with precipitation (Fig. 2a; $r^2 = 0.77$, $p = 0.049$), but negatively correlated with [NO_3^-] in the T1 stream sediments except in May 2007 (Fig. 2a and Table 2; $r^2 = 0.41$, $r = -0.48$, $p < 0.05$). At the T2 site, these correlations were not observed.

In riparian sediments, DEA was highest in December 2006 and was not affected by precipitation (Fig. 2b). DEA increased significantly with Ext. Org C concentrations at

Table 1. Average (s.d.) ($n = 20$) chemical properties and denitrification enzyme activities (DEA) of stream and riparian sediments of the Santa Fe River tributary in Florida, USA.

Site	pH	Dissolved O_2	NO_3^--N	NH_4^+-N	MBN	Ext. Org C	MBC	DEA
		mg·L^{-1}	mg N·kg soil^{-1}			mg C·kg soil^{-1}		mg N_2O-N·kg soil^{-1} day^{-1}
Stream sediment								
T1†	6 (0.4)‡	5.5 (1.5)	0.7 (0.1)b	4 (1)	14 (4)	35 (7)	1942 (356)	1.3 (0.2)c
T2	6.4 (0.8)	4.3 (1.4)	1.7 (0.1)a	3 (1)	11 (2)	32 (6)	1922 (326)	0.4 (0.2)d
Riparian sediment								
T1	6.5 (0.6)	4.7 (1.2)	0.7 (0.1)	10 (2)	21 (6)	87 (21)	4190 (947)	29 (7)e
T2	5.7 (0.4)	5.2 (1.5)	1.1 (0.3)	8 (1)	39 (13)	82 (16)	3083 (527)	7 (7)f

Note: †: Tributary 1, T1; Tributary 2, T2.

‡: Chemical properties and DEA were performed on sediment samples obtained in December 2006, and March, May, July and August 2007. Values in columns and stream type followed by different letters are significantly different ($p < 0.05$).

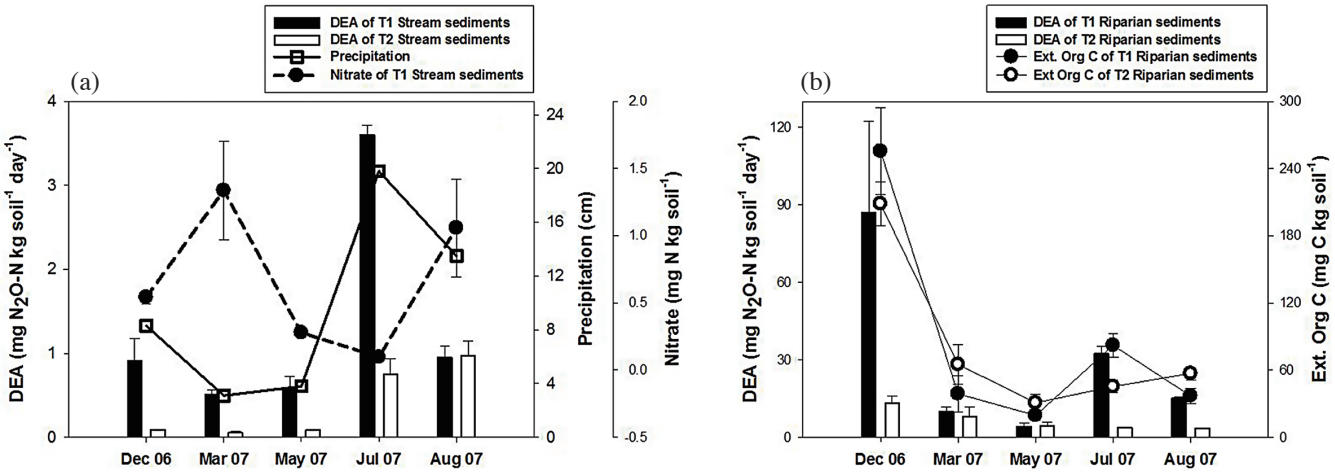

Fig. 2. Denitrification enzyme activities (DEA), precipitation and nitrate concentrations (NO_3^--N) in stream sediments (a), and extractable organic carbon concentrations (Ext. Org C) in riparian sediments (b) of the Santa Fe River tributaries, FL, USA. The sediment samples were collected from December 2006, and March, May, July, and August 2007. (n = 20; Abbreviation: Tributary 1, T1; Tributary 2, T2).

Table 2. Pearson correlations r between chemical properties and denitrification enzyme activities of stream and riparian sediments of the Santa Fe River tributary in Florida, USA.

Site	pH	NO_3^--N	NH_4^+-N	MBN	Ext. Org C	MBC
Stream sediment						
T1†	0.14	**-0.48**	-0.58	-0.03	-0.11	0.21
T2	-0.42	0.33	-0.2	-0.19	0.08	-0.24
Riparian sediments						
T1	-0.76	0.29	-0.15	0.08	**0.79**	0.31
T2	0.23	0.25	0.11	0.46	**0.67**	0.29

Note: †: Tributary 1, T1; Tributary 2, T2.
Chemical properties and DEA were performed using sediment samples obtained in December 2006, and March, May, July and August 2007. Statistically significant correlations ($p < 0.05$) are indicated by bold values.

both sites (Fig. 2b and Table 2; T1 site: $r^2 = 0.62$, r = 0.79, $p < 0.05$; T2 site: $r^2 = 0.45$, r = 0.67, $p < 0.05$). PD rates at the T1 site were positively correlated (Fig. 3) with Ext. Org C ($r^2 = 0.39$, $p < 0.05$) and MBC concentrations ($r^2 = 0.8$, $p < 0.05$). However, other chemical factors including pH, [NH_4^+], MBN, Ext. Org C, and MBC contents were not significantly correlated with DEA in both stream and riparian sediments (Table 2).

4. DISCUSSION

The DEA of the grassland stream sediments (the T1 site) were positively correlated with precipitation. This observation is contrary to the general observation that denitrification rate decreases as precipitation increases due to the increase in proportional ratios of water volume to sediment area in which denitrification occurs (Behrendt and Opitz 1999). However, precipitation can increase water pressure and enhance stream water penetration into sediments, thereby increasing the chance that water column NO_3^- and OM can reach denitrifying sites and enhancing denitrification rates in stream sediments (Morrice et al. 1997; Wroblicky et al. 1998). In addition, the elevated water table and flooding due to intense precipitation can easily create anaerobic conditions and increase the supply of OM, which is used by denitrifiers as electron donors. A previous study performed in various ecosystems including wetland ecosystems, forest and riverine riparian zones reported increased denitrification rates as the water table rose (Hill 1996; Freeman et al. 1997; Hefting et al. 2004; Peter et al. 2012). However, the decrease in water table due to extreme drought could increase nitrous oxide emissions produced by denitrification in nutrient poor sites (such as stream sediments). The reason is that the nitrate produced in the upper aerobic zones diffuses down into the anaerobic zone and is consumed by denitrifiers, and the reduction of nitrous oxide to nitrogen gas is incomplete when the oxygen content increases (Freeman et al. 1993; Dowrick et al. 1999). In addition, the increase in oxygen concentration due to water table reduction changes the denitrifying community structures and populations in water-saturated conditions, finally influencing denitrification rates (Liu et al. 2003; Kim et al. 2008). Thus, a water table change is an important factor that determines the denitrification rate but also nitrous oxide production and denitrifier community structures by enhancing nutrient exchange and creating aerobic and anaerobic conditions.

DEA was higher in the T1 stream sediments than in the T2 stream sediment, but [NO_3^-] was higher in T2 sediments than in T1 sediments. DEA was negatively correlated with

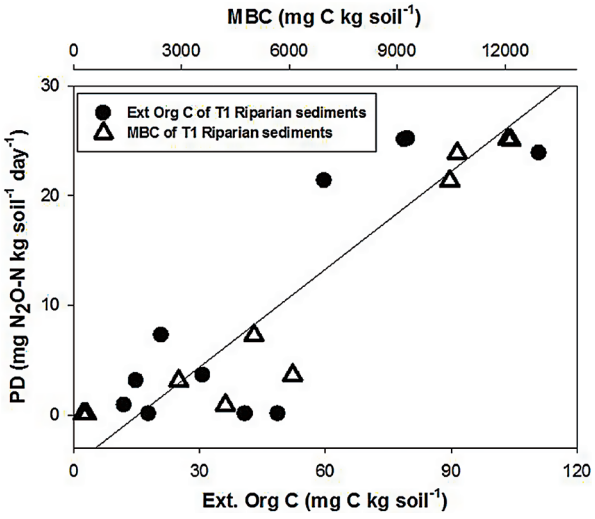

Fig. 3. Relationship between potential denitrification rates (PD), extractable organic carbon (Ext. Org C) and microbial biomass carbon (MBC) concentrations in riparian sediments of the Tributary 1 (T1) in the Santa Fe River, FL, USA. The sediment samples were collected from December 2006, and March, May, July and August 2007. (n = 20; Dark circle: Ext. Org C; Open triangle: MBC).

[NO_3^-] at the T1 site; this relationship suggests that denitrification removed NO_3^- from the T1 stream sediments. The T1 site is located near pasture ecosystems and is mainly surrounded by herbaceous vegetation, whereas the T2 site is located in the upstream region of a forest ecosystem and receives irrigated seepage water from a plant nursery that uses N fertilization and has a steeper slope gradient than the T1 site. Thus, the fast flow velocity due to the steeper slope of the T2 site could prevent NO_3^- and OM in the water from reaching the sediments, so that the NO_3^- easily flows downstream. Schipper and his colleague's (Schipper et al. 2004, 2005, 2010) research demonstrated that sediment denitrification rates increased when the water flow rate or hydraulic conductivity was low. This relationship emphasizes the importance of slow NO_3^- and OM diffusion through the immobile fraction where denitrification occurs. The T2 system also receives irrigated seepage water that contains inorganic N from fertilization, which increases [NO_3^-] at that site. DEA is lower in forest streams than in grassland streams because of the N uptake by woody plants in forest streams (Groffman et al. 1991), so this N from seepage water might cause the woody plants at the T2 site to consume more N than the herbaceous plants at the T1 site; i.e., N availability to denitrifying bacteria might be relatively reduced at the T2 site, thus imposing a limit of denitrification there.

The denitrification rate is increased by OM because denitrifiers are heterotrophic microbes that use OM as an electron donor and carbon source. The denitrification rate is positively correlated with OM concentrations ([OM]) in stream ecosystems (Hill et al. 2000; Inwood et al. 2007). Our results in the riparian sediments are consistent with these results and suggest that [OC] affects denitrification rates in riparian sediments. However, the T2 riparian sediments did not show a positive relationship between potential denitrification rates (PD) (when glucose was not added to samples) and [OC]. Litter C/N ratios were much higher in the forest streams (17) than grassland streams (12) (data not shown). This difference suggests that the T2 site might receive lower-quality of carbon materials than the T1 site. Previous research also demonstrated that denitrification rates were lower in riparian sediments treated with a high C/N ratio organic materials than in riparian sediments treated with a low C:N ratio organic materials, emphasizing the importance of carbon quality (Schipper et al. 1994; Bastviken et al. 2005; Hernadez and Mitsch 2007).

Unlike the results from the stream sediments, the DEA and Ext. Org C of the riparian sediments in this study were highest in the winter (December 2006). In general, available [OC] and denitrification rates are lower in winter because low temperature reduces microbial metabolic rates and because summer precipitation leaches nutrients (Sand-Jensen et al. 1988). However, the average temperature in December 2006 was 18°C, which was similar to that in March 2007 (19°C). Thus, the temperature effect on microbial activities might not have been significant at our research sites. In addition, falling leaves and detritus were washed from uplands during the summer and accumulated in riparian sediments. These decaying plant tissues might supply available carbon to denitrifiers, thereby allowing DEA to remain high during the winter.

DEA and PD were higher in riparian sediments than in stream sediments. Compared to the stream sediments, the riparian sediments were less disturbed by flowing water and retained more OM because they were located in the adjacent alluvial zones and received horizontal stream water flow. However, the stream water flow scours stream sediments and can therefore limit OM accumulation and reduce denitrification rates in stream sediments (Christensen and Sørensen 1988). In addition, bacteria living in the sediment are attached to the sediment particles in highly-structured assemblages such as biofilms (Costerton and Lappin-Scott 1995). Extracellular enzymes excreted by biofilm bacteria easily decompose non-labile carbon compounds such as lignin and increase the carbon quality (Lock 1993). The polyanionic polysaccharide matrix in the biofilm can capture OC and other nutrients from the stream water by ion exchange. Microbes use these resources as energy sources (Freeman and Lock 1995; Storey et al. 1999). Denitrification rates can be higher in biofilms than in other sites such as stream water due to the supply of a highly-degradable OC and nutrients from biofilms (Howard-Williams et al. 1989; Christensen et al. 1990; Mariñelarena and Giorgi 2001; Bastviken et al. 2003; Toet et al. 2003; Venterink et al. 2003). Furthermore, the development of biofilm is inversely proportional to stream flow velocity (Donlan 2002),

so riparian sediments with slow flow velocity might have more biofilms and higher denitrification rates than do stream sediments with relatively fast flow velocity.

In summary, our results indicate that denitrification in grassland stream sediments can strongly influence NO_3^- removal rates from agricultural streams. In forest streams NO_3^- removal by denitrification is limited by steep slopes that cause water to flow quickly, by N fertilization effects, and by the N uptake of trees. Our research indicates that environmental factors affected by different types of agricultural activities and vegetation might regulate denitrification rates and [NO_3^-] in agricultural stream ecosystems.

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