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Decomposition of cattle manure and colonization by macroinvertebrates in sediment of a wetland of the Middle Paraná River

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Abstract
Purpose The study was carried out in a wetland of the Middle Paraná River system, Argentina, in order to evaluate the processes associated with the decomposition of manure, which includes changes in chemical composition, nutrient release of manure, and colonization of invertebrates. We also compared the invertebrate assemblage that colonized manure with that present in the benthos of the wetland.
Materials and methods Nylon bags were filled with fresh cattle manure and anchored to the littoral zone of the wetland. Six bags were collected after 1, 2, 6, 14, 21, 28, 33, 55, and 79 days: three for invertebrate determination and the other three bags for determination of dry mass and chemical analyses. The nutrient content, cellulose, lignin, and total phenolic compounds of manure were determined. In addition, the leachate of manure was collected for nutrient analyses. Samples of the wetland benthic sediment were collected for benthic invertebrate determination and particle size analyses. Spearman rank correlation was used to evaluate the relationship between chemical compounds of manure and breakdown rate. Principal component analysis was used to explore invertebrate assemblage composition of manure and sediment during the experiment.
Results and discussion Limnodrilus, Dero, and *Chironomus* were dominant in the manure. Gatherer-collector was the dominant group in the manure, comprising almost 95 % of the total density of invertebrates. Breakdown rate was significantly related with nutrients, cellulose and total phenolics of manure. An ordination plot showed changes in invertebrate assemblages of manure and sediment samples over time.
Conclusions This study provides new insight on the importance of manure as a substrate for macroinvertebrate colonization. Cattle manure needs to be considered as a potential source of nutrients for aquatic systems and a substrate for invertebrate assemblages.

Keywords Benthic fauna · Chemical compounds · Functional feeding groups · Livestock

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1 Introduction

The use of aquatic systems by cattle is a worldwide issue (Scrimgeour and Kendall 2003; Steinman et al. 2003; Sigua 2010). Cattle preferentially occupy riparian habitats, which provide water, thermoregulation, shade, and nutritious forage (Roath and Krueger 1982; Pinchak et al. 1991). Cattle also get into aquatic systems, with direct and indirect impacts on water quality and biotic communities (Herbst et al. 2012; Mesa et al. 2015). Direct impacts include herbivory of aquatic vegetation, nutrient inputs via urine and fecal deposition, and trampling of the sediments (Archer and Smeins 1991; Collins et al. 1998). Indirect impacts include changes in macrophyte and algal species composition induced by nutrient loading and selective herbivory, which in turn can affect higher trophic levels that rely on these autotrophs for habitat, refugia and predation (Rader and Richardson 1994; Rader 1994). Such physical and chemical changes on the aquatic environment can alter the abundance and composition of invertebrate assemblages

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by modifying consumer resource dynamics (Townsend et al. 1997; Delong and Brusven 1998; Weigel et al. 2000).

In Argentina, since the late twentieth century, the expansion of soybean production has forced the relocation of cattle in wetlands for agriculture, constituting a threat to these ecosystems (PROSAP 2009). This situation resulted in a change in the cattle herd in floodplain systems, increasing from 160, 000 in 1997 to 1,500,000 during 2007 (Quintana et al. 2014).

The Paraná River drains the second largest watershed in South America after that of the Amazon, covering an area of 3.1×10^6 km^2. Most of its course is surrounded by a 10–50 km wide floodplain, resulting in a total area of 60,000 km^2. Because of their extensive areas of vegetation for foraging and high quality water supply, wetlands of the Paraná River system provide excellent conditions for cattle. Cattle spend a considerable amount of time grazing on riparian vegetation, and also within the wetlands to graze macrophytes and drink, where they defecate and urinate (Mesa et al. 2015). Because cattle graze in herds, this input of manure can be highly concentrated. Furthermore, the increase in water level during the high water period can result in the incorporation of considerable amounts of manure deposited in the marginal terrestrial area into the wetlands.

Several studies have suggested that manure from grazing cattle represents a source of nutrients to surface waters (Hongo and Masikini 2003; Kato et al. 2009; Mesa et al. 2015). Mesa et al. (2015) showed the increase in water nutrients during the presence of cattle in wetlands of the Middle Paraná River. However, the decomposition process of manure in aquatic systems, by which inorganic nutrients are incorporated into the sediment and water, is unknown.

Decomposition is an important process in ecosystem metabolism and a driving force in nutrient cycling as well as energy transfers (Webster and Benfield 1986; Wallace et al. 1997). This process is regulated by physical, chemical, and biological factors. The rates of decomposition and nutrient release are affected by the environment, the presence of decomposer organisms, and the chemical quality of organic material (Wallace and Webster 1996; Ardón et al. 2009). The latter is determined by an aromaticity of organic molecules and nutrient content of the decaying material (Ardón et al. 2006). In addition, total phenolics have been reported to affect breakdown rate in tropical streams (Stout 1989; Campbell and Fuchshuber 1995).

Macroinvertebrates can also play a key role in decomposition affecting decay rate, nutrient release, and primary production (Wallace et al. 2011; Pettit et al. 2012). Although several studies have assessed the importance of invertebrates in the decomposition process of manure in terrestrial systems (Wall and Lee 2010; Yoshitake et al. 2014), their role in the decomposition of manure in aquatic ecosystems is scarcely known. In order to gain insight into this, we aimed to evaluate the decomposition process of manure in a wetland of the Middle Paraná River, and includes (1) changes in chemical composition and nutrient release of manure during decomposition and (2) colonization of invertebrates in manure during this process. We also compare invertebrate assemblage that colonized manure with that present in the sediment of the wetland.

2 Materials and methods

2.1 Study site

This study was carried out in an isolated wetland (31° 39′ 39″ S, 60° 35′ 12″ W) of the Middle Paraná River located near Santa Fe city, La Capital, Santa Fe. Santa Fe is situated in the center of Argentina. This Province has a subtropical climate, with an average temperature of 32 °C during summer and 6 °C in winter. The studied wetland has an area of 47,000 m^2, with a maximum depth in the littoral zone of 1.0 m. The experiment was conducted from the 21st of November 2013 to the second of February 2014 during a low water period in summer season. Dominant emergent macrophytes in the experimental zone were *Ludwigia* sp., *Salvinia* sp., and *Azolla* sp., while *Elodea* sp., *Cabomba* sp., and *Ceratophyllum* sp. were the dominant submerged taxa.

2.2 Experimental setup

Fresh manure was collected over a 24-h period from an area adjacent to the studied wetland and immediately packaged in polyethylene bags. Sixty nylon bags of 20×30 cm were constructed with a mesh size of 1.5 mm and the lateral side with a coarse mesh of 2 mm. We used 1.5-mm mesh to prevent the rapid loss of small fragments of manure and a coarse side mesh in order to allow access for larger macroinvertebrates. Each bag was filled with 500 g of fresh dairy-cattle manure and tied to a 100-m long nylon rope in groups of six, separated by a distance of 70 cm in order to minimize disturbance during collection. Bags were anchored with iron stakes in the littoral zone of the wetland parallel to the shoreline, so that vegetation and substrate particle size composition were homogeneous along the transect. Bags were located in the littoral zone at a distance of 1 m to the shoreline on the bottom sediment of the wetland. This littoral zone was almost completely covered by floating macrophytes, so the exposure to waves in this zone was minimal. The experiment was finished when the remaining mass of dry manure was ~70 % in relation to the initial weight. We considered that a higher loss of organic matter would reduce significantly the substrate for macroinvertebrate colonization.

One group of six bags was collected after 1, 2, 6, 14, 21, 28, 33, 55, and 79 days, three for invertebrate determination, and the other three bags for determination of dry mass remaining and chemical analyses. In addition, three samples of bottom sediment for benthic invertebrates and one sample for particle size analyses were collected with a mud snapper (100 cm^2) on days 1, 28, and 79, along a transect parallel and immediately adjacent to the location of the bags. Physical and chemical variables such as depth, transparency (Secchi disk), temperature (standard thermometer), conductivity, dissolved oxygen, and pH (Hanna portable probes) were measured in situ at each collecting date.

2.3 Laboratory analyses

The three replicates of sediment and manure relative to each collection day were fixed with formaldehyde 4 % in the field. In the laboratory, invertebrates associated with these substrates were hand-picked under a stereoscopic microscope (magnification ×4) and preserved in 70 % alcohol for subsequent counting and identification. Samples relative to day 33 could not be used for analyses; concentration of formaldehyde in these samples was not sufficient to allow a proper conservation of specimens. The other three bags of manure were dried at 105 °C for 72 h and weighted with a Mettler electrobalance (0.1 mg accuracy) in order to determine the dry mass remaining and to be used for chemical analyses.

Taxonomic identifications of invertebrates relative to each replicate were made at the lowest taxonomic level possible (mostly genus or species) using the available keys (Brinkhurst and Marchese 1992; Domínguez and Fernández 2009; Trivinho-Strixino 2011). We classified taxa into functional feeding groups (FFGs) according to Merritt and Cummins (1996). In bottom sediment, percentage of sand (50–250 μm) and silt + clay (<50 μm) were determined in the laboratory according to Wentworth scale (1922).

2.4 Chemistry of manure leachate

In the field, bags of manure collected each day were put in a plastic tray immediately after they were removed from the water. The leachate of the three replicates (both dissolved constituents and any particles that passed through the mesh of the bags in a period of 5 min) was collected in different plastic bottles and stored on ice for subsequent nutrient analyses. The leachate (200–250 ml) was immediately filtered through Whatman GF/F glass-fiber filters. Filtered samples were refrigerated for determination of dissolved components within 24 h after sampling. Unfiltered leachate was frozen until their processing for determinations of total phosphorus (TP) and total nitrogen (TN). Soluble reactive phosphorus (SRP) was determined by the ascorbic acid method (Murphy and Riley 1962), nitrate + nitrite ($N\text{-}NO_3^- + N\text{-}NO_2^-$) by reduction of $N\text{-}NO_3^-$ with hydrazine sulfate and subsequent colorimetric determination of $N\text{-}NO_2^-$(Hilton and Rigg 1983), and ammonium ($N\text{-}NH_4^+$) by the indophenol blue method (Koroleff 1969). Total P was determined by digestion with nitric and sulfuric acids followed by SRP determination (Koroleff 1972) and TN by digestion with potassium persulfate in an alkaline medium followed by $N\text{-}NO_3^- + N\text{-}NO_2^-$ determination (APHA 2005).

2.5 Chemistry of dry manure

Dried manure relative to the three replicates of each day of collection was rough ground, homogenized, and fine ground to pass through a 1 mm sieve for chemical analyses. Total P was determined on 1 g of finely ground dried sample that was combusted at 550 °C in a muffle furnace for 4 h. The remaining ash was suspended in 3 M HCl (Andersen 1976) and filtered for the subsequent colorimetric analysis of SRP. Total N of manure was determined by Kjeldhal digestion.

Phenolic compounds were extracted with 95 % (*v/v*) methanol for 24 h in darkness (Ainswoth and Gillespie 2007). The extract was centrifuged, and the supernatant was used for determining the phenolic compounds by the Folin–Ciocalteu reagent (Box 1983). Structural compounds (cellulose and lignin) were estimated with sequential Neutral Detergent/Acid Detergent digestion on an Ankom fiber analyzer (ANKOM Fiber Technologies, Fairport, New York, USA; Goering and Van Soest 1970).

2.6 Data analyses

2.6.1 Macroinvertebrate assemblage structure

Macroinvertebrate assemblages associated with manure were described by three assemblage metrics: density (number of individuals $gram^{-1}$ of dry remaining manure), taxonomic richness (total number of taxa), and Shannon diversity index. Density of invertebrates in manure and sediment (number of individuals m^{-2}) were averaged for subsequent analyses.

2.6.2 Breakdown rate of manure

Breakdown rate for each day of the experiment (k) was determined by adjusting the data from manure remaining dry mass to the exponential negative model: $W_t = W_0\, e^{-kt}$ (Olson 1963). For degree day calculations ($k_{\text{degree day}}$), we substituted in this equation the time (t) by the thermal sum in degree days on the collection day (Cummins et al. 1989). As water and air temperature were correlated ($R > 0.90$, $P < 0.001$), we used the mean daily air temperature for this analysis.

2.6.3 Statistical analyses

Spearman rank correlation was used in order to evaluate the relationship between chemical compounds of leachate and dry remaining manure with k. The nonparametric Kruskall Wallis test was used to evaluate differences in invertebrate assemblage metrics, chemical variables of manure leachate, and dry manure during the period of the experiment (R program, version 3.1.1, package Stats).

Principal component analysis (PCA) was used to explore the invertebrate assemblage composition of manure and sediment during the experiment. The three replicates of invertebrate density of manure and sediment relative to each collection day were averaged and standardized for this analysis (Legendre and Gallagher 2001). ANOSIM (using the Bray–Curtis similarity index) was conducted to detect differences in the structure of macroinvertebrate assemblages between sediment and manure samples. These analyses were conducted with R program, version 3.1.1, package Vegan.

3 Results

Values of physical and chemical variables of the wetland taken in the zone where the experiment was conducted are shown in Table 1. The TP and TN contents in water during the experimental period varied between 0.29–0.70 and 0.60–3.00 mg l^{-1}, respectively. Taking into account these values, this wetland was classified as eutrophic (OECD 1982; Knowlton and Jones 1997). Water temperature varied between 22 and 29 °C. Dissolved oxygen showed a minimum value (2.3 mg l^{-1}) on day 33, whereas the maximum value (11.8 mg l^{-1}) was reported on day 28. Sediment particle size composition was composed of 50 % silt + clay and 50 % sand.

3.1 Invertebrate assemblage composition of manure and sediment

A total of 31 taxa colonized manure during the experiment (Table 2). Oligochaeta reached the highest density (relative density >90 %) followed by Chironomidae (relative density >5 %). Diptera (other than Chironomidae), Collembola, Nematoda, and Coleoptera constituted <2 % of total density. *Limnodrilus hoffmeisteri* (35 %), *Dero (Aulophorus) lodeni* (18 %), and *D. (A.) borellii* (10 %) were the dominant taxa. *Chironomus* sp. dominated among Diptera (Chironomidae). Gatherer-collector was the dominant FFG that colonized manure, accounting for over 95 % of individuals. Predators, collector-filterers, and shredders represented as small proportion of the total invertebrate fauna (<2 %) (Table 2).

A total of 23 macroinvertebrate taxa were found in the benthos of the wetland (Table 2). Oligochaeta was the dominant group (density >65 %), followed by Chironomidae (12.4 %) and Collembola (10.6 %). Diptera (other than Chironomidae), Nematoda, Coleoptera, and Crustacea constituted <10 % of total density. *Aulodrilus piguetti* (22 %) and *Dero (Dero) multibranchiata* (14 %) were dominant in the sediment samples. *Tanytarsus* sp. dominated among Chironomidae (relative density 33 %). Gatherer-collector was the dominant FFG in the sediment (density >70 %), followed by collector-filterer (20 %) and predator (9 %) (Table 2).

3.2 Changes in assemblage metrics during decomposition

Taxa richness, density, and diversity in manure varied significantly during the experiment (Kruskall–Wallis test, $P < 0.05$). Colonization of manure by invertebrates was fast, reaching a total of 12 taxa at the day 2 of immersion. Richness was lower in the day 2 compared to the first day ($P < 0.05$), and then varied between 8 and 12 during the studied period (Fig. 1). The Shannon diversity was significantly higher at day 2 and 21 ($P < 0.05$), with lower values at day 79 in comparison with the beginning of the experiment ($P < 0.05$). The density of macroinvertebrates colonizing manure was high at day 55 compared to the first days of the experiment ($P < 0.05$) (Fig. 1).

3.3 Ordination analysis

The PCA axis 1 and 2 explained 25 and 19 % of the variation in the structure of invertebrate assemblages of manure and sediment during the experiment. Axis 1 separated samples over time;

Table 1 Mean values and standard deviation (SD) of physical and chemical variables of the Middle Paraná River wetland measured during the 79 day experimental period

Environmental variables	1	2	6	14	21	28	33	55	79
Dissolved oxygen (mg l^{-1})	6.65	5.0	6.0	5.0	5.5	11.8	2.3	2.2	2.4
Transparency (cm)	50	50	40	25	15	29	11	52	60
Depth (cm)	72	72	60	80	82	71	59	52	62
Water temperature (°C)	26.2	26.6	22.8	26.7	24.5	28	28.7	26	27
Conductivity (μS cm^{-1})	64	76	90	64.2	60.7	71.1	79	67.7	51.5
pH	7.5	8.8	7.1	7.6	6.4	8.7	7.8	7.9	6.9
Total dissolved solids (ppm)	29	30	32	32.3	30.3	35.5	39.8	34	25.8
TN (mg l^{-1})	0.63 (100)	0.66 (43)	0.80 (109)	0.74(102)	2.92(377)	2.51 (153)	2.13(205)	1.84 (114)	1.82 (121)
$N\text{-}NH_4^+$ (mg l^{-1})	0.54 (454)	0.04 (3.5)	0.10 (19.1)	0.006 (3.9)	0.04 (26)	0	0.04 (1.2)	0.1 (108)	0.02 (3.2)
$N\text{-}NO_3^- + N\text{-}NO_2^-$ (mg l^{-1})	0.17 (122)	0.001 (3)	0.06 (9.2)	0.01 (9.2)	0.03 (29)	0	0	0.04 (1.2)	0.05 (1.8)
TP (mg l^{-1})	0.68 (113)	0.60 (197)	0.29 (63)	0.26 (503)	0.64 (243)	0.42 (274)	0.38 (353)	0.30 (21)	0.40 (50)
SRP (mg l^{-1})	0.03 (1.9)	0.02 (1.1)	0.02 (2.6)	0.03 (1.4)	0.03 (6.6)	0.04 (3.2)	0.07 (3.3)	0.04 (5)	0.03 (0.6)

Table 2 Occurrence of taxa in any one of the three replicates of manure and sediment (marked cross) throughout the experiment

Taxa	Abbreviation	FFG	Manure									Sediment		
			1	2	6	14	21	28	33	55	79	1	2	3
Ablabesmyia sp.	*Abl*	P						x					x	
Alotanypus sp.	*Alo*	CF								x				
Beardius sp.	*Bea*	CG					x					x		x
Chironumus sp.	*Chi*	CG		x	x	x	x			x			x	
Goeldichironomus sp.	*Goe*	CG												x
Labrundinia sp.	*Lab*	P									x			x
Onconeura sp.	*Onc*	CG										x		
Parachironomus sp.	*Par*	CG		x										x
Polypedilum sp.	*Pol*	CG									x			x
Tanytarsus sp.	*Tan*	CF		x		x	x						x	x
Aulodrilus piguetti	*A.pig*	CG												x
Bothrioneurum sp.	*Bot*	CG			x		x			x				x
Dero (Aulophorus) borellii	*D.bor*	CG			x	x	x	x	x	x	x		x	x
Dero (Aulophorus) furcatus	*D.fur*	CG				x		x						
D. (Aulophorus) lodeni	*D.lod*	CG		x		x	x	x		x	x			
D. (Dero) botrytis	*D.bot*	CG		x										
D. (Dero) cooperi	*D.coo*	CG				x	x		x	x				
D. (Dero) evelinae	*D.eve*	CG		x		x	x			x				
D. (Dero) digitata	*D.dig*	CG												x
D. (Dero) multibranchiata	*D.mul*	CG		x	x	x	x	x				x		x
D. (Dero) nivea	*D.niv*	CG				x						x		x
D. (Dero) obtusa	*D.obt*	CG						x		x				x
D. (Dero) sawayai	*D.saw*	CG		x						x				x
Limnodrilus hoffmesteri	*L.hof*	CG		x	x	x	x		x	x	x	x		x
L. udekemianus	*L.ude*	CG									x			
Pristina americana	*P.ame*	CG				x				x	x			
P. synclites	*P.syn*	CG						x			x			
Stylaria sp.	*Sti*	CG					x							
Slavina isochaeta	*S.iso*	CG												x
Trieminentia corderoi	*T.cor*	CG				x								
Nematoda	Nem	P		x				x				x		
Colembolla	Col	CF		x										
Odonata	Odo	P												
Copepoda	Cop	CF												
Ephydridae	*Eph*	P	x	x										
Chaoborus sp.	*Cha*	P						x						
Stratiomyidae	*Str*	CG							x					
Curculionidae	*Cur*	P					x					x		
Culicidae	*Cul*	CG											x	
Hebetancylus sp.	*Heb*	CF										x		

Assignation to functional feeding groups (FFG) is also shown

samples relative to the first days of the experiment were placed in the negative side. However, while manure samples of the last days of the experiment were in the positive side, later sediment samples were in the negative side. Axis 2 differentiated manure samples of the middle day of the experiment in the positive side.

A total of 35 taxa were present in the center of the ordination, demonstrating the similarity in assemblage composition of manure and sediment samples (Fig. 2). This finding was in agreement with ANOSIM, showing a lack of a significant difference in macroinvertebrate

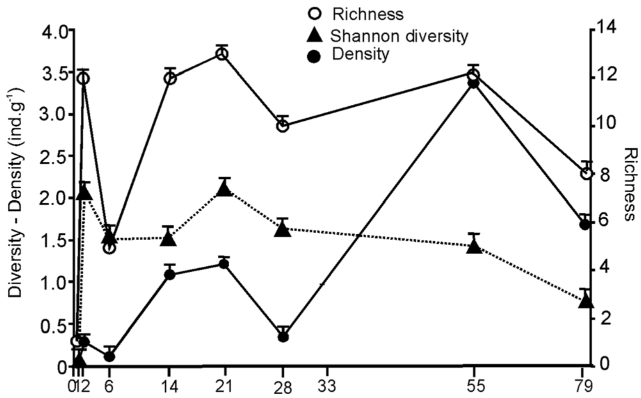


Fig. 1 Invertebrate metrics associated with manure during the experiment. *Lines* above the figures represent the standard errors of the mean (three replicates of manure per sample day)

assemblage structure between manure and sediment samples (ANOSIM, $R = -0.164$, $P > 0.05$).

3.4 Nutrient concentration in leachate and dry manure

Nutrient concentrations varied significantly during the experiment (Kruskall–Wallis test, $P < 0.05$). Concentration of TP in dry manure and TP and SRP in manure leachate were significantly higher during the first 6 days of the experiment ($P < 0.05$), decreasing thereafter (Fig. 3a). Total N in dry manure decreased significantly in the second day of the experiment ($P < 0.05$), increasing TN in leachate during this period (Fig. 3b). Then, TN in dry manure increased between days 6 and 21 ($P < 0.05$), and in the final days of the experiment (Fig. 3b). N-NH_4^+ was higher during the first 6 days ($P < 0.05$), decreasing at the end of the experiment. In addition, N-NO_3^- + N-NO_2^- was below the analytical detection limit at the beginning, increasing at day 28 ($P < 0.05$, Fig. 3c). Cellulose and total phenolics decreased, whereas lignin increased during decomposition (Fig. 3d).

3.5 Decomposition rate and dry mass remaining

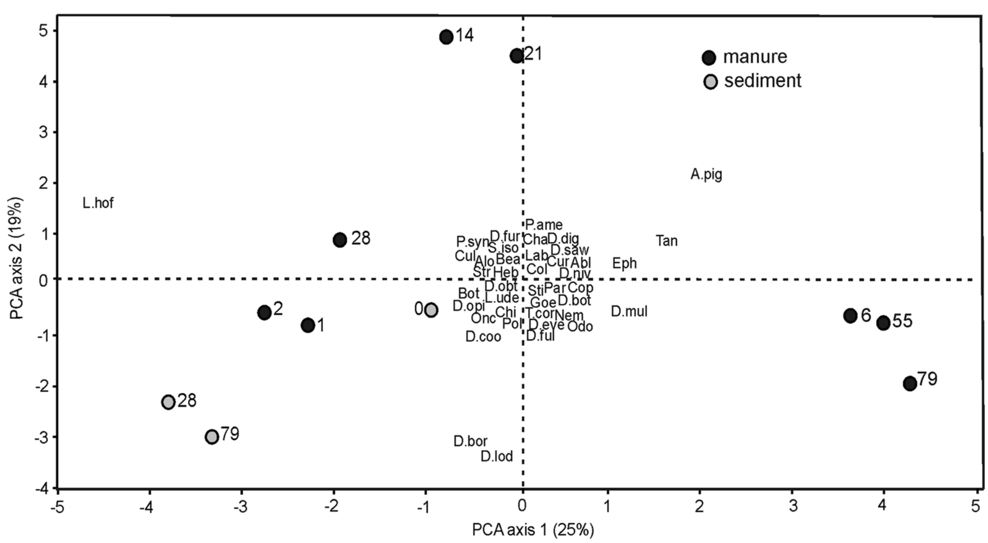
During the experiment, k varied between 0.01 and 0.08 whereas $k_{\text{degree day}}$ was 0.0004. Higher values of k were detected during the first 6 days. Then, k decreased, remaining relatively constant to the end of the experiment (Fig. 4). The mass remaining showed a constant decrease through the studied period (Fig. 4). The percentage mass remaining at the end of the experiment (day 79) was 25 %. The estimated time for 50 % decomposition of manure was 25 days. The 50 % of dry weight remaining of manure approximates 750 degree-days, corresponding to the fast category according to Cummins et al. (1989).

Values of k were significantly positively correlated with concentration of SRP, TP, and TN in manure leachate ($P < 0.05$) and negatively related with N-NO_3^- + N-NO_2^- ($P < 0.01$) (Table 3). In addition, TP, cellulose, and phenolic compounds content of dry manure was positively correlated with decomposition rate ($P < 0.01$) (Table 3).

4 Discussion

This study provides new insight on the importance of manure as a substrate for the colonization of macroinvertebrates. Manure could represent a trophic resource for benthic invertebrates which may forage on the fine and coarse particulate organic matter that make up the manure (Del Rosario et al. 2002). Likewise, algae and biofilm that colonize this substratum can be another source for primary consumers (Del Rosario et al. 2002). Moreover, even species that do not exploit manure as a trophic resource, can use it as habitat and refuge to avoid predators (Ramseyer and Marchese 2009). Once manure gets into the aquatic system, it would introduce patches in the bottom sediment that are rapidly colonized by benthic taxa. Taxa such as *Limnodrilus, Dero*, and *Chironomus* contributed >

Fig. 2 Principal component analysis (PCA) ordination of macroinvertebrate assemblages associated with manure and sediment. For each axes, the eigenvalue is shown in parenthesis



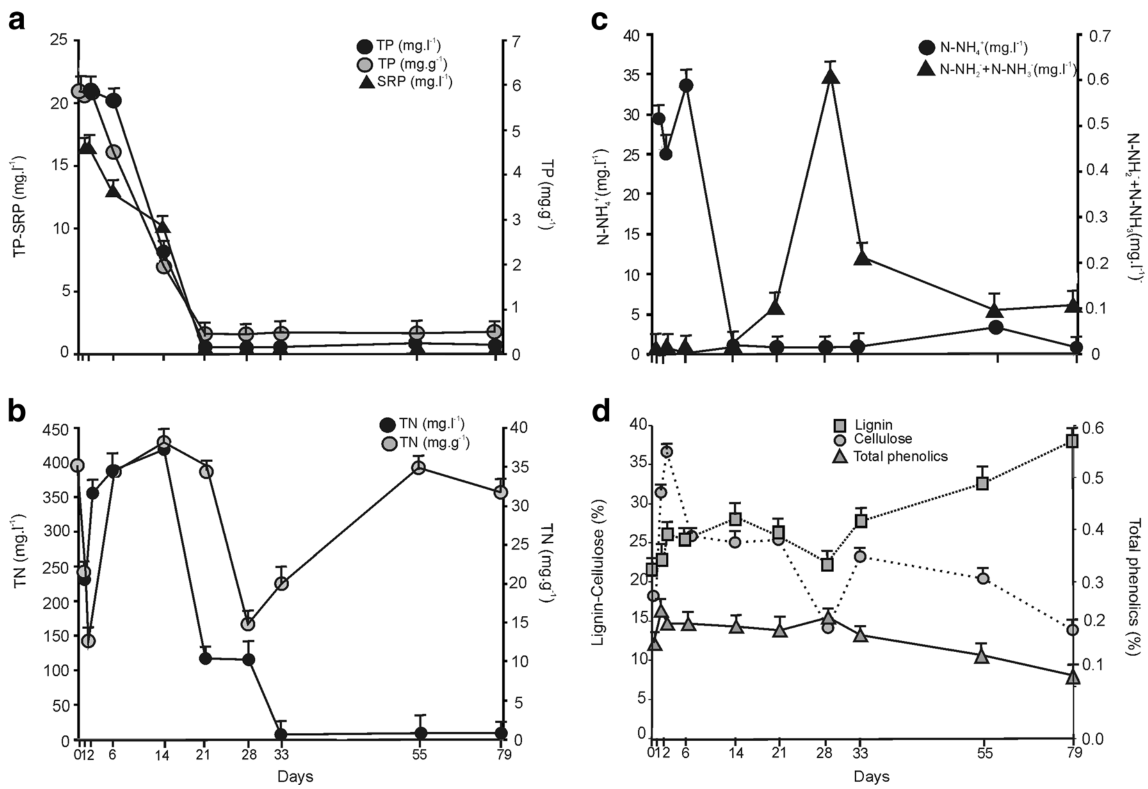


Fig. 3 Variation in chemical compounds of dry manure and nutrients in manure leachate during the experiment. *Lines* above the figures represent the standard errors of the mean (three replicates per sample)

80 % of total density of macroinvertebrates in manure, whereas in benthic samples these taxa represented < 35 % of total density. Manure could represent a rich organic substrate susceptible to be colonized only by tolerant taxa. *Limnodrilus, Dero*, and *Chironomus* have morphological adaptations (presence of anal gills, higher concentration of hemoglobin; Marchese and Paggi 2003) to survive in substrates of high organic content, with high bacterial activity and low levels of dissolved oxygen (Marchese and Ezcurra de Drago 2006).

The relative density of gatherer–collectors was higher in manure than in the benthos of the wetland, representing almost 95 % of total density of invertebrates in manure. In the rumen of cattle, plant materials are partially decomposed, thus providing a high proportion of fine particulate organic matter in manure available for gatherer-collectors. Gatherer–collector is the most important feeding group in Paraná River floodplain whereas scrapers and collector filterers represent a lower proportion of the total density of invertebrates (Ezcurra de Drago et al. 2007). The absence of shredders in samples of manure and sediment

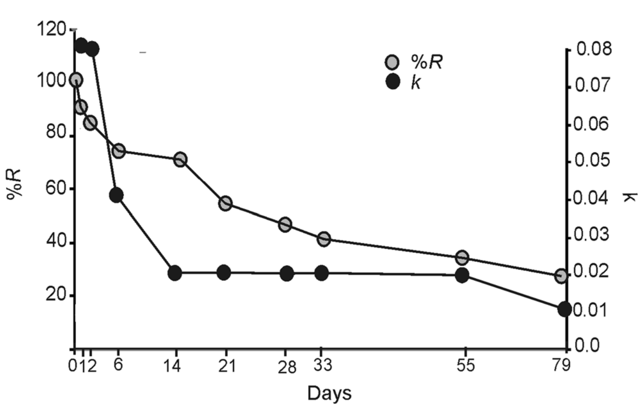


Fig. 4 Changes in the percentage of mass remaining (%*R*) and breakdown rate (k_{day}) during the experiment

Table 3 Correlation (Spearman rank coefficient) between chemical compounds of manure leachate, dry remaining manure, and decomposition rate (*k*)

Chemical compounds		*k*	
		R	*P*
Manure leachate	N-NH_4^+ (mg l^{-1})	0.66	ns
	N-NO_3^- + N-NO_2^- (mg l^{-1})	−0.76	<0.01
	SRP (mg l^{-1})	0.98	<0.001
	TP (mg l^{-1})	0.70	0.03
	TN (mg l^{-1})	0.76	<0.01
Dry manure	TP (mg g^{-1})	0.85	<0.01
	TN (mg g^{-1})	0.56	ns
	Lignin (%)	−0.54	ns
	Cellulose (%)	0.79	<0.01
	Phenolic compounds (%)	0.94	<0.001

ns no significant

was in agreement with that reported for tropical aquatic systems (Wantzen and Wagner 2006) and for the Paraná River basin (Ezcurra de Drago et al. 2007). Consequently, microbes likely control leaf litter breakdown and subsequent release of nutrients in these systems (Franken et al. 2005; Reid et al. 2008).

Invertebrate density in manure showed an increase with time during the study period. Probably, during the last phase of decomposition, the conditions within manure bags were more suitable for macroinvertebrate colonization, in comparison with those at the first phases of decomposition. Perhaps, the low dissolved oxygen concentration at the beginning of the experience, caused by a greater amount of organic matter decomposition, could act as a restrictive factor for invertebrate colonization. Likewise, during early phases of decomposition, high concentrations of ammonia could be expected, causing toxic conditions for most of species (EPA 2013).

Breakdown rates of manure reported here ($k = 0.01$–0.08) are higher than values reported for tree litterbag studies in tropical streams elsewhere (see Wantzen et al. 2008). The estimated time for 50 % decomposition was higher than those reported for macrophytes in lentic systems of the Paraná River floodplain (Neiff and Neiff 1990; Pagioro and Thomaz 1999; Poi de Neiff et al. 2006). Manure, as a partially decomposed material, added to its rapid microbial and invertebrate colonization would result in rapid decomposition. Furthermore, the eutrophic condition of the wetland where the experiment was conducted and high water temperature related to the season of this experiment were factors that would contributed to the rapid breakdown of manure (Webster and Benfield 1986; Irons et al. 1994). Furthermore, the breakdown rate based on degree days was low compared to those reported in temperate regions (Cummins et al. 1989). This lower value is related to the higher values of temperature registered during the study period, determining higher degree day values compared with those registered in temperate zone (Cummins et al. 1989). In this subtropical region, in contrast with temperate ecosystems, invertebrate shredders are scarce and, thus, litter breakdown is carried out mainly by bacteria. As bacterial activity requires higher temperatures than shredders (Irons et al. 1988), then more degree-days would be necessary to decompose the same amount of litter. As a result of this, the estimation of breakdown rates based on degree days should be lower for bacteria-driven process.

The lack of waves in the wetland and the scarce water movement in the benthic zone as a result of the wind protection by macrophytes result in a minimum loss of fine material through the net of the bags. In addition, decomposition would be the most important process driving the loss of organic matter during the experiment. Furthermore, the high transparency and coverage of submerged and floating macrophytes able to retain sediments in the zone of the experiment would indicate a low sedimentation on bags.

In addition, manure breakdown was significantly related to nutrient content, total phenolics, and cellulose. This finding was in accordance with other studies about leaf decomposition (Ardón et al. 2009; Li et al. 2009), showing that toughness and nutrient content controls breakdown rates in tropical aquatic systems. Recalcitrant carbon compounds have large three-dimensional, complex structures that can be broken down only by specialized enzymes, thus making them metabolically more costly for microbes (Gessner and Chauvet 1994; Hutchens and Benfield 2000). As decomposition proceeds, the proportion of lignin in manure increases as microbes preferentially metabolize other chemical fractions such as cellulose (Melillo et al. 1983).

The high initial TN and TP content of manure may promote greater decomposition by microbial communities as well as leachate of nutrients and fast incorporation into the aquatic environment (Stohlgren 1988; Mathuriau and Chauvet 2002). Nutrients were lost by leachate at the beginning of the experiment. This corresponds to the first decomposition phase of manure that includes initial loss of dry weight, attributed to the rapid leachate of the soluble organic and inorganic nutrients. Initially, SRP was the main fraction of TP lixiviated and then its proportion with respect to TP decreased substantially. $N\text{-}NH_4^+$ was also leached mostly at the beginning of the study. This nitrogen form constitutes the end product of the ammonification of organic nitrogen performed by heterotrophic bacteria. Ammonification occurs without changing the N oxidation state (−3) and therefore $N\text{-}NH_4^+$ is the first inorganic N form produced during the mineralization of organic matter. The produced $N\text{-}NH_4^+$ could be converted to $N\text{-}NO_3^-$ by bacterial oxidation. Consequently, when the concentrations of organic N and $N\text{-}NH_4^+$ decreased, an increase in $N\text{-}NO_3^-$ was observed, which was the main form of dissolved inorganic N leached at the end of the study. In addition, the observed decrease in TN in dry manure until the second day could reflect the rapid leaching of organic and inorganic compounds. Before the sixth day, the increase in TN in dry manure could be related to the immobilization caused by an accumulation of microbial biomass and products of microbial activity, production of mucopolysaccharide and bacteria exudes (Rice 1982; Rice and Hanson 1984). This increase was similar to that observed in other studies investigating macrophytes breakdown in tropical regions (Pagioro and Thomaz 1999).

5 Conclusions

The results of this work provide evidence of the role of manure for invertebrate assemblages and nutrients concentrations in wetlands. The input of manure into the aquatic system would not only introduce nutrients by decomposition but also introduce new habitats to be colonized by benthic invertebrates. There were three invertebrate taxa dominant in manure. More studies are necessary in order to evaluate the utility of these taxa in bio-assessment for gaging impacts associated with cattle disturbance.

In floodplain systems like the Paraná River, cattle could play an important role in the transference of nutrients from the riparian and terrestrial vegetation to water and bottom sediment of aquatic systems, thus linking terrestrial primary production and aquatic secondary production. The high nutrient concentration of manure needs to be taken into account to evaluate the effect of cattle on eutrophication of wetlands. Variables such as stocking density should be considered in order to avoid undesirable effects on nutrients inputs.

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