Changes in soil carbon in response to flooding of the floodplain of a semi-arid lowland river

Darren S. Baldwin[1,4], Warren L. Paul[2,5], Jessica S. Wilson[3,6], Tara Pitman[3,7], Gavin N. Rees[1,8], and Annaleise R. Klein[3,9]

[1]Murray–Darling Freshwater Research Centre and CSIRO Land and Water, La Trobe University, Wodonga, Victoria 3689 Australia
[2]Department of Environmental Management and Ecology, La Trobe University, Wodonga, Victoria 3689 Australia
[3]Murray–Darling Freshwater Research Centre, La Trobe University, Wodonga, Victoria 3689 Australia

Abstract: Soil C is a key factor influencing soil health and, by inference, ecosystem condition. In arid and semi-arid regions, soil moisture limits accumulation and decomposition of soil C. On floodplains, soil moisture can come from rainfall events or periodic flooding. The effect of flooding on soil C decomposition has received some attention in the literature but mostly through the study of agricultural systems or mesocosms. Field studies of actual floods are not common. We measured soil C response to a managed flood on a semi-arid lowland river floodplain in southeastern Australia before and after inundation at control (unflooded), floodplain (low hydraulic energy), and flood runner (high hydraulic energy) sites. At control sites, soil C changed little from before to 16 d after the onset of the flood. At flooded sites, most measures of soil C (total soil organic matter [floodplain sites only], permanganate-oxidizable C, persulfate-extractable C, water-extractable C [flood runner sites only], microbial C, and root material) were lower 16 d after than before the flood. However, the activity of key hydrolytic exo-enzymes associated with soil C decomposition did not change. Following the initial decline in soil C at the flooded sites, soil properties remained essentially constant for the remainder of the flood. Upon flood recession, slight increases in C pools occurred at the flood runner site (possibly associated with the formation of debris dams) but not at the floodplain or control sites. The changes in soil C pools after flood recession were at least an order of magnitude lower than those observed at the same location during more extended flooding. We argue that the loss of soil C associated with short-duration floods may adversely affect ecosystem condition of lowland river floodplains. Managed floods should be of long enough duration (months) to promote the growth of submerged and emergent aquatic macrophytes on the floodplain because aquatic macrophyte production is an important source of soil C after flood recession in lowland river floodplains.

Key words: decomposition, flooding regime, reciprocal provisioning model, water management

Soil C is a key factor influencing soil health (Arias et al. 2005). It contributes to soil character and fertility (Stevenson 1994), and by inference, to ecosystem condition. Soil C is a basal resource for microbial communities and many of the soil meiofauna, and nutrients essential for plant growth are released as it decomposes and mineralizes. Soil C also influences soil physiochemical properties including water retention, aggregation, pH buffering, and cation exchange capacity (Stevenson 1994), and it may impart resilience to semi-arid lowland river floodplains under conditions of water deficit caused by river regulation, irrigation diversions, and climatic drought (Colloff and Baldwin 2010).

Soil C can be classified on the basis of reactivity (Arias et al. 2005). The passive, refractory fraction can remain in soil for centuries to millennia (and is the focus of the current interest in the long-term sequestration of C in the soil profile); the slightly more reactive fraction has turnover times on the order of years to decades, and the reactive fraction has short turnover time (hours to months) and is the principal energy source for soil respiration and microbial activity (Wilson et al. 2011).

In most terrestrial ecosystems, fixation (and decomposition) of soil organic matter is mediated by soil moisture, mostly through rainfall. In arid and semi-arid regions, soil moisture is a limiting factor (Ogle and Reynolds 2004, Morton et al. 2011). This limitation has led to formulation of the *pulse–reserve* model for ecosystem functioning in arid and semi-arid ecosystems (Noy-Meir 1973). In the pulse–reserve model, rainfall events are triggers for pulses of plant growth and subsequent formation of reserves of

E-mail addresses: [4]darren.baldwin@csiro.au; [5]w.paul@latrobe.edu.au; [6]j.wilson@latrobe.edu.au; [7]tara.pitman@lls.nsw.gov.au; [8]gavin.rees@csiro.au; [9]a.klein@latrobe.edu.au

DOI: 10.1086/681009. Received 17 June 2013; Accepted 3 September 2014; Published online 26 February 2015.
Freshwater Science. 2015. 34(2):431–439.

organic C in the soil (Ogle and Reynolds 2004, Morton et al. 2011).

Soil moisture and, by extension, soil C in lowland river floodplain ecosystems in arid and semi-arid regions also can be affected by periodic flooding. The effect of flooding on soil C fixation and decomposition has received some attention in the literature, but most earlier research was directed toward constructed agricultural systems, especially rice paddies (Bossio and Scow 1995, Patrick and Reddy 1976). More recently, the effect of flooding on soil C decomposition has been studied in mesocosms (Unger et al. 2009, Wilson et al. 2011). Mesocosms are useful because they specifically allow for replication and tight control of climatic variables (e.g., temperature, light, and flooding frequency) and the types of measurements that can be taken and their frequency are not limited by site access. However, mesocosm studies usually rely on moving soil from the field to a laboratory setting and can suffer from experimental artifacts, such as edge effects. Thus, results of these studies may be difficult to interpret at larger spatial and temporal scales (Spivak et al. 2011), and complementing results from mesocosm studies with results from actual floods is beneficial. However, floods are difficult to study. They are often unpredictable, so pre-inundation data collection can be problematic. Site access may be difficult or impossible once a flood has arrived, so studies of real flooding events on soil C dynamics are rare. An exception is a study by Valett et al. (2005), who used a managed flood to explore biogeochemical processes, including floodplain soil metabolism, on a semi-arid floodplain. They found that soil respiration rates (a measure of soil C mineralization) increased 2 orders of magnitude relative to unflooded controls within 5 d of flooding and that this high rate of metabolic activity persisted for at least 2 wk.

Baldwin et al. (2013) used a space-for-time experimental approach and showed that flooding can affect soil C reserves. Submerged and emergent aquatic macrophytes that grew on the floodplain during inundation provided a substantial subsidy of C to the floodplain after flood recession. In particular, reserves of soil C were substantially higher immediately after flood recession than before flooding. Moreover, these reserves could persist for years. Therefore, flooding was able to increase the rates of C mineralization and C fixation. This subsidy is the basis of the *reciprocal provisioning* model of floodplain functioning.

An environmental watering event (artificial flooding specifically to improve the condition of the floodplain and, particularly, vegetation health) in July 2009 at Yanga National Park on the lower Murrumbidgee River in southeastern Australia provided a unique opportunity to study changes in soil C in the field during a flood. Sufficient notice was given prior to flooding that pre-inundation data could be collected, and the nature of the floodplain was such that it was accessible during the flood. We used this event as an opportunity to measure changes in soil properties (particularly soil C species) after flooding at replicate sites with different potential flow velocities and compared these data with data from unflooded control sites.

Based on results of earlier studies (Valett et al. 2005, Wilson et al. 2011), we hypothesized: 1) an initial rapid increase in enzyme activity in the soils upon inundation, followed by 2) an increase in soil respiration rate, which would lead 3) initially to a decline in bioavailable soil C fractions, but 4) eventually to a substantial increase in soil organic C pools as aquatic macrophyte growth was stimulated by flooding.

METHODS

Study location

We worked in the river red gum (*Eucalyptus camaldulensis* Denh.) forest of Yanga National Park (Yanga) on the lower Murrumbidgee River floodplain near Balranald, New South Wales, Australia (lat 34.35°S, long 143.84°E; Fig. 1). The region has a mean annual rainfall of 320 mm/y and net evaporation of 1615 mm/y (Kingsford and Thomas 2004), but in 9 of 12 y between 1997 and 2009, the region received below-average rainfall (Bureau of Meteorology 2010), and only 192 mm of rain was recorded in the park in 2009.

The environmental watering event commenced in July 2009. Water levels in the adjacent Murrumbidgee River were elevated by raising the height of Redbank Weir (1 in Fig. 1). Water was diverted into the park by opening the 1AS regulator (2 in Fig. 1), which allowed water to flow down a flood runner and enter Two Bridges Swamp. Closure of a 2nd set of regulators at Two Bridges Swamp (3 in Fig. 1) caused the water to be distributed through the park by a series of flood runners (Fig. 1).

Sampling

We used a stratified, 2-stage cluster-sampling design. We stratified by locations that would undergo flooding with potentially different flow velocities. We chose 3 sites in an area where water would spread out across a relatively wide region of the floodplain (floodplain sites), 3 sites immediately downstream where the flow would be constricted into a single flood runner, and 3 control sites where flooding was not predicted to occur (Figs 1, S1). We sampled 0.5 to 6 h before inundation (T_0); after 16 (T_{16}), 28 (T_{28}), and 48 d (T_{48}) of inundation; and on day 102 (T_{102}), when the flood had receded and the sites were dry.

We collected each sample by removing the relatively sparse leaf litter and then taking the top 5 to 10 cm of soil with a hand auger. On each sampling occasion, we took 5 replicate soil samples at each site from within a 5-m radius. We scooped subsamples of each replicate soil sample into 70-mL sterile polypropylene screw-top jars and froze them immediately for subsequent analysis of enzyme activity. We pooled approximately equal amounts of soil from

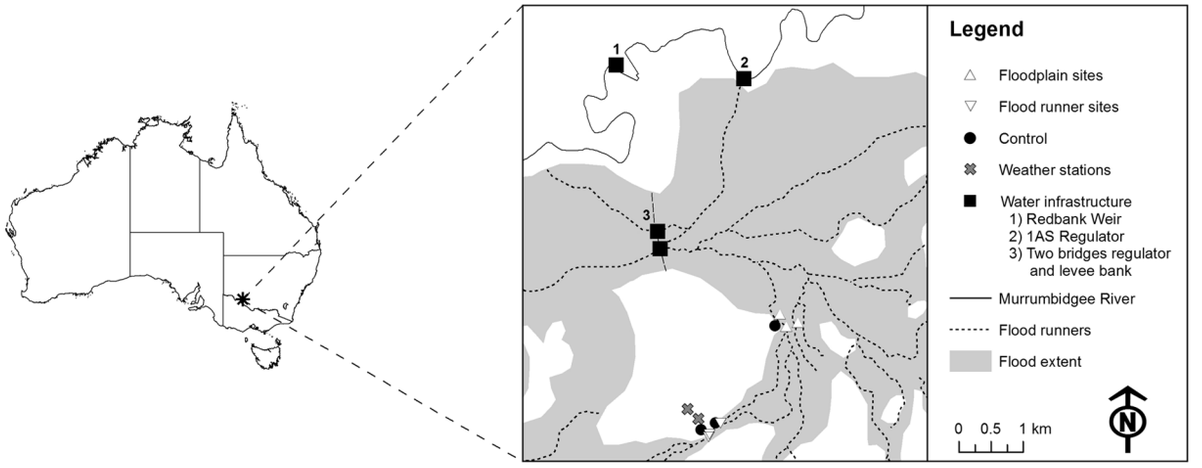

Figure 1. Map of the control and flooded sites and the infrastructure used to create the artificial flood in the lower Murrumbidgee River.

each replicate at each site into 1 preweighed 500-mL polypropylene screw-topped jar and kept it at 4°C for gravimetric analysis of soil moisture. We placed the reminder of each soil sample in 1-L Ziploc® plastic bags and stored them at 4°C for subsequent chemical analysis.

Chemical analyses

We measured soil mass before drying within 8 h of sampling. We measured soil moisture and soil bulk density gravimetrically by oven-drying soil samples at 105°C overnight. Moisture content was calculated as the difference in soil mass before and after drying (Gardner 1986). We measured salinity (electrical conductivity $[EC]_{1:5}$) and pH on soil dried at 50°C, ground to 2 mm, and rehydrated to a 1 : 5 soil-to-water ratio (Rayment and Higginson 1992). We measured $EC_{1:5}$ before pH to prevent contamination of the sample with electrolyte from the pH electrode.

We estimated mass of coarse root material in soil samples dried at 5°C by weighing the dry material retained by a 10-mm sieve and expressed the mass as g/kg dry soil. We estimated total soil organic matter ($TOM_{<10mm}$) as loss on ignition (550°C for 2 h) of ~5 g of soil that passed through the 10-mm sieve and had been ground and sieved to 2 mm.

We measured permanganate-oxidizable C ($KMnO_4$-C) with a modified version of the method of Blair et al. (1995). We combined the equivalent of ~1 g dry mass of field-moist soil (exact mass recorded) and 25 mL of 33.3 mM $KMnO_4$ in 50-mL centrifuge tubes. We shook samples and a blank (containing reagent but no soil) for 1 h at 20°C and centrifuged the sample for 5 min at 1400*g*. We diluted 1 mL of supernatant to a final volume of 25 mL with Milli-Q water (EMD Millipore, Darmstadt, Germany) and measured absorbance (565 nm) with a Varian Cary spectrometer (Agilent Technologies, Palo Alto, California). We calculated the amount of C in the samples as described by Blair et al. (1995) by assuming that 1 mmol $KMnO_4$ oxidizes 9 mg of C.

We prepared samples for measuring water-extractable (H_2O-C) and potassium sulfate-extractable C (K_2SO_4-C) with a method similar to that described by Cookson et al. (2008). For H_2O-C, we shook 10 g of field-moist soil and 40 mL of Milli-Q water on an orbital shaker for 1 h, centrifuged the sample for 20 min at 18,000*g*, filtered the supernatant through 0.45-µm pore-size cellulose acetate syringe filters (Bonnet Equipment, Sydney, Australia), and froze the supernatant prior to analysis. For K_2SO_4-C we extracted 10 g of field-moist soil in 40 mL of 0.5 M K_2SO_4 (1 : 4 soil solution ratio) for 1 h at 20°C, centrifuged the sample for 10 min at 1400*g*, filtered the supernatant through 0.45-µm pore-size cellulose acetate syringe filters (Bonnet), and froze the supernatant prior to analysis. We prepared samples for measurement of microbial biomass C by fumigation extraction (Brookes et al. 1985a, b). We placed ~10 g (exact mass was recorded) of field-moist soil in a glass beaker in a desiccator and fumigated with ethanol-free chloroform at 20°C for 24 h, then removed the chloroform, and evacuated the desiccator at least 4 times to remove all chloroform from the samples. We extracted the fumigated soil in 40 mL of 0.5 M K_2SO_4 (1 : 4 soil solution ratio) for 1 h at 20°C, centrifuged the sample, and filtered and stored the supernatant frozen prior to analysis. We used an extraction-efficiency multiplier (K_{ec}) of 0.35 for general soils (Howarth and Paul 1994). We analyzed H_2O-C, K_2SO_4-C and microbial biomass C (MB-C) by the heated-persulfate oxidation method (APHA 2005) using a 1010 total C analyzer (IO Analytical, College Station, Texas). We calculated MB-C by the method of Howarth and Paul (1994) by subtracting the K_2SO_4-C from the C measured in the fumigated samples.

We measured organic acids (formate, acetate, butyrate, and propionate) by ion chromatography (IC) after pooling the 5 replicate samples taken at each site. We combined five 1-g samples (1 g from each replicate) of soil that had been dried at 50°C and passed through a 2-mm sieve. We

extracted the combined sample in 15 mL of Milli-Q water for 30 min on an orbital shaker, centrifuged the sample at 18,000*g*, and filtered it through a 0.22-μm pore-size nitrocellulose membrane (EMD Millipore, Darmstadt, Germany). We acidified the filtrate with 0.5 mL of 5 mM perchloric acid and removed residual carbonate and bicarbonate by bubbling with instrument-grade air for 15 min. We used a Metrohm Compact 761 IC, equipped with a Metrohm Metrosep organic acids 250/7.8 column with Metrosep Organic Acids Guard/4.6 (Metrohm, Herisau, Switzerland), using 0.5 mM perchloric acid eluent, run in conjunction with a Metrohm MSM module for suppression with 10 mM lithium chloride, to improve signal-to-noise ratio.

We measured esterase, leucine aminopeptidase, alkaline phosphatase, W- and β-glucosidase, and β-xylosidase activities with a modified 96-well microplate fluorometric enzyme assay (Marx et al. 2001). We prepared methylumbelliferyl-linked (MUB) substrates in 5.0 mM buffer (phosphate pH 7 for esterase and leucine aminopeptidase, bicarbonate pH 8.1 for the other enzymes), to give a final concentration of 500 μM. We weighed thawed and homogenized soil samples from each replicate at each site (~0.2 g) and pooled them in 400 mL of Milli-Q water. We placed the slurries in a sonicating water bath for 2 min to break up the soil macroaggregates (Stemmer et al. 1998, De Cesare et al. 2000). We set up enzyme assays with 150 μL soil suspension and 150 μL substrate and incubated them for 2.5 h. We measured production of fluorescent products at 20°C with a Fluoroskan Ascent plate reader (Labsystems, Vantaa, Finland). We corrected initial enzyme activities for quenching and used an MUB standard curve to calculate enzyme activities.

Climatic and soil data

We situated 2 automatic monitoring stations for soil moisture, soil temperature, and rainfall adjacent to the study site on a noninundated part of the floodplain. Each station measured volumetric soil moisture with capacitance probes (EC-5; Decagon Devices, Inc., Pullman, Washington) at 5-, 15-, and 30-cm depth; soil temperature (S-TMB-M0XX; Onset Computer Corp., Bourne, Massachusetts) at 5 cm depth; and rainfall (S-RGB-M002; Onset Computer Corp.) on an hourly basis with a HOBO H21-001 weather station (Onset Computer Corp.). We inserted the soil moisture and temperature sensors into the solid face of a trench while ensuring that the sensors contacted the soil with minimal disturbance to the surrounding soil structure. We present the data as the daily average of the 2 stations.

Data analysis

We corrected all measurements for field moisture content and expressed them on a dry mass basis. We converted pH data to concentration of protons before analysis. We used SigmaPlot (version 11; Systat Software Inc., San Jose, California) to calculate descriptive statistics (arithmetic mean and standard error) after pooling the results from the 5 replicates at each of the 3 control, floodplain, and flood runner sites. We used R for all other data analyses (R Project for Statistical Computing, Vienna, Austria; Pinheiro et al. 2013) after $\log_{10}(x)$-transformation of all variables except pH to approximate a normal distribution. We examined residual diagnostic plots to check that the transformed data were consistent with the assumptions of the analysis of variance (ANOVA) and linear mixed-effects (LME) models. We assessed the statistical significance of changes in enzyme activity and volatile acids with ANOVA because the samples were composited by site before measurement. We used LME to assess statistical significance of changes in the concentration of each of the C fractions, pH, and EC (Pinheiro and Bates 2000). We included a random effect for site in the LME models to accommodate the 2-stage cluster-sampling design. We included fixed effects for location, time, and the location × time interaction. Location × time means and 95% confidence intervals were derived from the fitted models.

RESULTS

Climatic data

Approximately 40 mm of rain, >20% of the measured rain in the park in 2009, fell in the study area within the 14 d before sampling (Fig. 2). An additional 20 mm of rain fell on the day after initial sampling. This rain made roads in the region impassable and delayed the 1st post-flood sampling event. Soil moisture at the unflooded weather stations peaked after this last rainfall event and then fell during the remainder of the study until reaching a minimum of

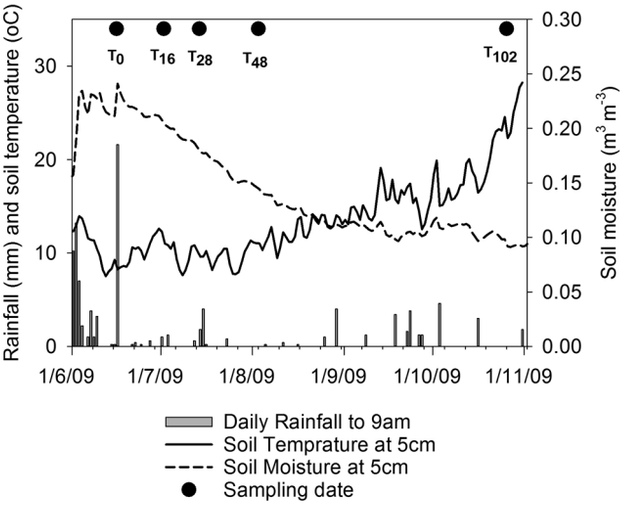

Figure 2. Rainfall, soil temperature at 5-cm depth, and soil moisture at an unflooded site adjacent to the study sites. Sampling dates are shown at the top of the graph. Dates are formatted d/m/yy.

~10 m^3/m^3 (Fig. 2). Soil temperature rose from just below 10°C before sampling to almost 30°C at the end of the sampling program (Fig. 2).

Preflood soil properties

With the exception of soil $EC_{1:5}$, none of the chemical variables measured differed among control, floodplain, and flood runner sites immediately before flooding ($p > 0.05$). $EC_{1:5}$ was slightly higher at the floodplain sites than at the control and flood runner sites (Table 1).

Initial changes in soil properties (T_0 to T_{16})

Soil properties at control sites did not change between T_0 and T_{16} (Table 1). At the floodplain sites, $TOM_{<10mm}$, MB-C, $KMnO_4$-C, K_2SO_4-C, root biomass, and $EC_{1:5}$ decreased ($p < 0.05$) and soil moisture increased ($p < 0.05$), but enzyme activities did not change. At the flood runner sites, MB-C, $KMnO_4$-C, K_2SO_4-C, H_2O-C, root-biomass, and $EC_{1:5}$ declined ($p < 0.05$), but enzyme activities did not change.

Changes in soil properties during the flood (T_{16} to T_{48})

Except for an increase in acetate at the flood runner and floodplain sites ($p < 0.05$), no further changes occurred in any soil C species or enzyme activity throughout the rest of the flooded period (data not shown). Acetate concentrations increased at floodplain and flood runner sites and reached a maximum on T_{48} (Tables 1, 2), whereas acetate concentrations at unflooded control sites did not change during the flooded period.

Changes in soil properties during flood recession (T_{48} to T_{102})

Acetate and $EC_{1:5}$ increased significantly between T_{48} (the last time the sites were sampled while inundated) and T_{102} (~20 d after flood recession; Table 2) at all sites. MB-C and root biomass increased in flood runner and floodplain sites but not in unflooded control sites. Total soil organic matter, $KMnO_4$-C, and K_2SO_4-C increased at flood runner sites, but not at floodplain sites (Table 2).

Soil properties at flooded vs unflooded sites at T_{102}

$TOM_{<10mm}$, MB-C, $KMnO_4$-C, and acetate were higher at flood runner than at floodplain or unflooded control sites at T_{102} ($p < 0.05$), but did not differ between floodplain and unflooded control sites. K_2SO_4-C was higher at control and flood runner sites than at floodplain sites ($p < 0.05$). Flooding followed by drying did not affect root biomass or enzyme activity.

DISCUSSION

Flooding

We described a conceptual model of C dynamics in floodplain soils in response to flooding in our earlier work

Table 1. Concentration of key analytes (mean ± SE) at control, floodplain, and flood runner sites immediately before flooding (T_0) and after 16 d of inundation (T_{16}). Bold indicates a significant difference between T_0 and T_{16} ($p < 0.05$) calculated by analysis of variance (enzyme activity) or linear mixed-effects model (all other analytes). Arrows indicate the direction of change. EC = electrical conductivity, MB = microbial biomass. See Methods for explanation of extractable C.

Variable	Unflooded control		Floodplain		Flood runner	
	T_0	T_{16}	T_0	T_{16}	T_0	T_{16}
pH	6.38	6.43	6.03	**6.55** ↑	6.06	6.26
EC (μS/cm)	143 ± 21	120 ± 11	217 ± 49	**80 ± 3** ↓	148 ± 13	**101 ± 7** ↓
$TOM_{<10mm}$ (%)	9.5 ± 1.3	8.9 ± 0.5	10.7 ± 1.53	**6.4 ± 0.3** ↓	8.9 ± 0.4	7.2 ± 0.6
MB-C (mg/kg)	1480 ± 247	1090 ± 44	1540 ± 220	**540 ± 63** ↓	1400 ± 150	**790 ± 110** ↓
$KMnO_4$-C (mg C/kg)	2.5 ± 0.3	2.0 ± 0.1	2.8 ± 0.5	**0.83 ± 0.02** ↓	3.1 ± 0.5	**1.1 ± 0.02** ↓
K_2SO_4-C (mg C/kg)	50 ± 2	47 ± 2	56 ± 21	**8.8 ± 0.7** ↓	49 ± 8	**17 ± 1.1** ↓
H_2O-C (mg C/kg)	71 ± 4	53 ± 4	58 ± 18	42 ± 9	52 ± 3	**32 ± 2** ↓
Root material (g/kg)	1.9 ± 0.07	**6.9 ± 1.7**↑	3.6 ± 2	**0.11 ± 0.11** ↓	2.6 ± 0.4	**1.4 ± 0.7** ↓
Acetate (mg/kg)	7.94 ± 0.74	7.37 ± 0.64	8.65 ± 0.44	8.89 ± 0.67	7.82 ± 0.60	8.24 ± 1.29
Esterase activity (nmol g^{-1} h^{-1})	2220 ± 530	1770 ± 390	1670 ± 690	2330 ± 580	1850 ± 330	1640 ± 360
Leucine aminopeptidase activity (nmol g^{-1} h^{-1})	404 ± 103	315 ± 67	304 ± 133	415 ± 116	332 ± 55	287 ± 63
Phosphatase activity (nmol g^{-1} h^{-1})	488 ± 99	411 ± 56	377 ± 113	485 ± 117	475 ± 21	401 ± 38
α–glucosides activity (nmol g^{-1} h^{-1})	77 ± 13	68 ± 11	46 ± 21	71 ± 23	69 ± 14	50 ± 16
β–glucosides activity (nmol g^{-1} h^{-1})	278 ± 76	218 ± 21	178 ± 99	245 ± 105	220 ± 41	146 ± 41
β–xylosides activity (nmol g^{-1} h^{-1})	76 ± 17	62 ± 14	51 ± 22	73 ± 24	69 ± 10	54 ± 15

Table 2. Concentration of key analytes (mean ± SE) at control, floodplain, and flood runner sites during flooding (T_{48}) and after flood recession (T_{102}). Bold indicates a significant difference between T_{48} and T_{102} ($p < 0.05$) calculated by analysis of variance (enzyme activity) or linear mixed-effects model (all other analytes). Arrows indicate the direction of change. EC = electrical conductivity, MB = microbial biomass. See Methods for explanation of extractable C.

Variable	Control		Floodplain		Flood runner	
	T_{48}	T_{102}	T_{48}	T_{102}	T_{48}	T_{102}
pH	6.55	6.30	6.54	6.12	6.32	**5.98** ↓
EC (μS/cm)	93 ± 23	**162 ± 27** ↑	99 ± 7	**184 ± 22** ↑	103 ± 8	**243 ± 14** ↑
$TOM_{<10mm}$ (%)	7.3 ± 1.4	6.9 ± 0.5	8.2 ± 0.8	8.1 ± 0.8	6.8 ± 0.5	**11.2 ± 0.9** ↑
MB-C (mg/kg)	890 ± 190	730 ± 180	460 ± 50	**1070 ± 110** ↑	680 ± 60	**1600 ± 130** ↑
$KMnO_4$-C (mg C/kg)	1.6 ± 0.4	1.2 ± 0.4	0.91 ± 0.1	1.6 ± 0.2	0.94 ± 0.2	**2.6 ± 0.5** ↑
K_2SO_4-C (mg C/kg)	49 ± 12	119 ± 14	11 ± 1	22 ± 5	13 ± 2	**44 ± 15** ↑
H_2O-C (mg C/kg)	60 ± 13	**95 ± 11** ↑	48 ± 14	40 ± 6	43 ± 3	57 ± 10
Root material (g/kg)	1.6 ± 0.7	7.7 ± 4.8	0.0 ± 0.0	**1.9 ± 0.5** ↑	0.86 ± 0.7	**6.7 ± 3** ↑
Acetate (mg/kg)	7.97 ± 1.62	**26.50 ± 2.43** ↑	24.40 ± 2.81	**44.20 ± 4.99** ↑	19.80 ± 0.39	**49.50 ± 5.52** ↑
Esterase activity (nmol g^{-1} h^{-1})	2030 ± 360	1430 ± 400	2260 ± 420	2000 ± 530	2120 ± 790	2330 ± 560
Leucine aminopeptidase activity (nmol g^{-1} h^{-1})	363 ± 76	242 ± 74	393 ± 79	370 ± 100	370 ± 139	430 ± 105
Phosphatase activity (nmol g^{-1} h^{-1})	463 ± 88	312 ± 79	486 ± 86	468 ± 69	474 ± 126	549 ± 70
α–glucosides activity (nmol g^{-1} h^{-1})	65 ± 20	41 ± 20	73 ± 22	63 ± 13	64 ± 31	74 ± 14
β–glucosides activity (nmol g^{-1} h^{-1})	243 ± 85	114 ± 55	226 ± 74	242 ± 69	184 ± 81	282 ± 74
β–xylosides activity (nmol g^{-1} h^{-1})	63 ± 18	42 ± 20	69 ± 21	66 ± 14	68 ± 30	77 ± 15

(Wilson et al. 2011). This model was based on a mesocosm experiment on soils from a floodplain river (Ovens River, southern Australia) that is in a Mediterranean climatic zone and undergoes periodic short-term floods (the longest flood recorded was 22 d). Our current study was done on a large floodplain in a semi-arid climatic zone where, when floods do occur, they last for many months (Kingsford and Thomas 2004). Our data indicate some consistencies between the 2 studies.

In the Ovens River study, the maximum mineralization rate occurred after ~20 d of flooding, after which it was postulated that the rate would decline (MacGregor 2006). We did not measure mineralization rates in the Murrumbidgee River floodplain. Instead, we measured changes in concentration of C species over time in response to flooding. In 16 d, total soil organic matter ($TOM_{<10mm}$ + root biomass) declined by ~20% at flood runner sites and 40% at floodplain sites but <1% at unflooded control. $KMnO_4$-C (a measure of easily oxidized and, hence, microbially available C; Lefroy et al. 1993) and K_2SO_4-C (a measure of C loosely adsorbed to clay; Wang et al. 2003) decreased at flooded sites. Some of this lost organic matter could have been leached from the soil profile, but we think it unlikely that this pathway contributed much to the loss of C. Other investigators have shown that flooded soil tends not to release much C (O'Connell et al. 2000), and H_2O-C concentrations in the soils in our study were ~4 orders of magnitude smaller than the total amount of C lost in the 16 d after inundation. We argue that the amount of H_2O-C was so low in our samples that mobilization of C via dissolution could not have been a major pathway for transport. Therefore, one of the most likely causes of C loss from the soils after flooding would be C mineralization through soil microbial and micro- and meiofaunal respiration. Therefore, both studies indicate a substantial initial decline in soil C/increase in C mineralization after inundation.

After the initial decline, the loss of C from the soil slowed substantially. We observed no change in the concentrations of $TOM_{<10mm}$, root biomass, $KMnO_4$-C, or K_2SO_4-C in the flood runner or floodplain sites between T_{16} and T_{48}. The most likely explanation for the decline in C mineralization rate is that all of the readily available C had been consumed. The more recalcitrant fractions, like lignin, would break down much more slowly. Humification, which would lead to a decline in soil C bioavailability, also may have been occurring (Prescott 2010). In an earlier study, we showed a substantial shift in the 3-dimensional fluorescence signals between dry and inundated soils (Wilson et al. 2011). Non-inundated soils had spectral maxima that we attributed to fulvic-like and amino acid-like substances. After 24 d of inundation, the amino acid-like peak had disappeared and was replaced with one attributed to humic-like substances.

MB-C declined in all sites between T_0 and T_{16}, but the decline was substantially greater at flooded than at unflooded sites. Some loss of MB-C would be expected on initial flooding because osmotic shock would rupture cell

membranes (Baldwin and Mitchell 2000), but inundation also would have favored colonization by opportunistic species (Wilson et al. 2011). We also speculate that the first postflooding sampling period may have coincided with the onset of anoxia in the soils and the loss of obligate aerobes.

The exo-enzyme activity measured at the beginning of our study is similar to activities measured in noninundated floodplain soils (Wilson et al. 2011). In our study, inundation had no apparent effect on exo-enzyme activity. However, in other studies, exo-enzyme activities increased rapidly immediately after flooding before declining to preflood levels within a few days (Burns and Ryder 2001, Wilson et al. 2011). Therefore, the 16-d delay in postflooding sampling may have caused us to miss short-term changes in exo-enzyme activity.

Flood recession

A number of changes occurred during flood recession. $TOM_{<10mm}$ increased at the flood runner site but not the control or the floodplain sites. This difference might be attributable to the formation of litter dams around coarse woody debris and deposition of material from upstream at the flood runner sites. Thus, flooding may be important in rearranging particulate organic C on the floodplain, even if it is not the principal vector for moving particulate organic matter from the floodplain into lowland rivers (Valett et al. 2005).

$KMnO_4$-C increased at flood runner and floodplain sites, as did MB-C, acetate, and root material at floodplain sites. With the exception of acetate, none of these variables changed at unflooded control sites over the same period. These soil properties might have been responding to the transition from a fully saturated, potentially anaerobic soil profile to a partially saturated, potentially aerobic soil profile in a manner similar to the pulse of microbial activity and plant growth observed in arid and semi-arid terrestrial zones after rainfall (the pulse–reserve model; Ogle and Reynolds 2004, Collins et al. 2008, Morton et al. 2011).

Impact of the flood on soil C

We observed a rapid decline in soil C stores in response to inundation. Some of this C was returned to the soil upon flood recession either via lateral movement of material across the floodplain or an increase in some C pools through processes that appear similar to those described in the pulse–reserve model of arid and semi-arid land functioning (Ogle and Reynolds 2004). The net result was either no change (floodplain sites) or a slight increase (flood runner sites) in most soil C pools relative to those at unflooded control sites. Based on the reciprocal provisioning model, we would have predicted substantially more soil C in the flooded sites after flood recession. We showed previously that flooding can make a substantial contribution to soil C at this location (Baldwin et al. 2013), but most of the C was derived from the growth of submerged and emergent macrophytes, which can contribute up to 800 g dry mass/kg of aboveground biomass, up to 50 g/kg root and rhizome biomass, and ~400 g/kg $TOM_{<10mm}$ (Baldwin et al. 2013). This source of organic C can then fuel terrestrial food webs (Iles et al. 2010) and microbial soil processes for years following flood recession and is the basis of the reciprocal provisioning model of lowland river floodplain functioning (Baldwin et al. 2013).

The changes in soil C pools in our study are at least an order of magnitude lower than changes reported by Baldwin et al. (2013). Root biomass changed at most by only several grams of C/kg, and $TOM_{<10mm}$ changed by only a few tens of grams of C/kg during the course of study. The principal difference between the 2 studies was the extent of macrophyte growth. Aquatic macrophytes did grow during our study, but not to any great extent (as evidenced by change in root biomass). The extent of macrophyte growth depends on a number of interacting factors, including species, period and depth of inundation, water and air temperature, and nutrient status (Newman et al. 1997, Sharma et al. 2008, Silva et al. 2009). In the absence of substantial macrophyte growth during the flood phase, inundation has the potential to remove soil organic matter from the floodplain and, thus, could negatively affect floodplain functioning and resilience, especially to extended periods of drought (Colloff and Baldwin 2010).

Implications for arid and semi-arid lowland river floodplain management

We showed that inundation results in the rapid loss (mineralization) of floodplain soil C. This rapid loss of soil C is consistent with earlier mesocosm studies of floodplain soil inundation (Wilson et al. 2011). Given the relationship between soil C and overall soil health (Arias et al. 2005) and, by inference, floodplain ecosystem condition and function (Colloff and Baldwin 2010), continued loss of soil C without replacement (mostly through growth of aquatic macrophytes during extended periods of inundation) could result in a decline in the overall condition of the floodplain ecosystem.

Most of the world's rivers are regulated. As such, many floodplains worldwide are considered under threat, principally because they have been isolated to some extent from their rivers (Tockner et al. 2010). On the lower Murrumbidgee floodplain (where Yanga National Park is situated), flood extent, frequency, and duration have all declined substantially because of river regulation and over-extraction of water (Kingsford and Thomas 2004, Page et al. 2005). To compensate for this lack of flooding, artificial floods, like the one in our study, are being implemented at many floodplain sites worldwide. When designing artificial floods in arid and semi-arid lowland river floodplains (i.e., in ecosystems that rely on aquatic macrophyte growth during inundation as a source of soil C), consideration should be given

to implementation of floods of sufficient duration (months) and timing to facilitate the growth of aquatic macrophytes. More important, to conserve soil C in arid and semi-arid lowland river floodplains, repeated floods of a short duration (weeks) without any longer-duration flooding should be avoided when possible.

ACKNOWLEDGEMENTS

This study was funded by the Australian Government's Commonwealth Environmental Research Facilities (CERF) Significant Projects Program. DSB and GNR acknowledge the support of CSIRO Water for a Healthy Country National Research Flagship. We thank John Pengelly for the H_2O-C and MB-C analyses, James Maguire and Paul Childs from the New South Wales Office of Environment and Heritage for useful discussion on flooding regimes, Todd Wallace (Murray–Darling Freshwater Research Centre) and Matt Colloff (CSIRO Land and Water Flagship) for general discussions on the overall project, and the rangers at Yanga National Park for assistance in accessing the park. We also thank Matt Colloff for commenting on an earlier version of this manuscript.

LITERATURE CITED

APHA (American Public Health Association). 2005. Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Arias, M. E., J. A. Gonzalez-Perez, F. J. Gonzalez-Vila, and A. S. Ball. 2005. Soil health—a new challenge for microbiologists and chemists. International Microbiology 8:13–21.

Baldwin, D. S., and A. M. Mitchell. 2000. The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river–floodplain systems: a synthesis. Regulated Rivers: Research and Management 16:457–467.

Baldwin, D. S., G. N. Rees, J. S. Wilson, M. J. Colloff, K. L. Whitworth, T. L. Pitman, and T. A. Wallace. 2013. Provisioning of bioavailable carbon between wet and dry phases in a semi-arid floodplain. Oecologia (Berlin) 172:539–550.

Blair, G. J., R. D. B. Lefroy, and L. Lisle. 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Australian Journal of Agricultural Research 46:1459–1466.

Bossio, D. A., and K. M. Scow. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied and Environmental Microbiology 61:4043–4050.

Brookes, P. C., J. F. Kragt, D. S. Powlson, and D. S. Jenkinson. 1985a. Chloroform fumigation and the release of soil-nitrogen: the effects of fumigation time and temperature. Soil Biology and Biochemistry 17:831–835.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985b. Chloroform fumigation and the release of soil-nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17:837–842.

Bureau of Meteorology. 2010. Climate statistics for Australian locations: Balranald. Bureau of Meteorology, Canberra, Australia. (Available from: http://www.bom.gov.au/climate/averages/tables/cw_049002.shtml)

Burns, A., and D. S. Ryder. 2001. Response of bacterial extracellular enzymes to inundation of floodplain sediments. Freshwater Biology 46:1299–1307.

Collins, S. L., R. L. Sinsabaugh, C. Crenshaw, L. Green, A. Porras-Alfaro, M. Stursova, and L. H. Zeglin. 2008. Pulse dynamics and microbial processes in aridland ecosystems. Journal of Ecology 96:413–420.

Colloff, M. J., and D. S. Baldwin. 2010. Resilience of floodplain ecosystems in a semi-arid environment. Rangeland Journal 32: 305–314.

Cookson, W. R., D. V. Murphy, and M. M. Roper. 2008. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. Soil Biology and Biochemistry 40:763–777.

De Cesare, F., A. M. V. Garzillo, V. Buonocore, and L. Badalucco. 2000. Use of sonication for measuring acid phosphatase activity in soil. Soil Biology and Biochemistry 32:825–832.

Gardner, W. H. 1986. Water content. Pages 493–544 *in* A. Klute (editor). Methods of soil analysis. Part 1. Physical and mineralogical methods. Soil Science Society of America, Madison, Wisconsin.

Howarth, W. R., and E. A. Paul. 1994. Microbial biomass *in* R. W. Weaver, J. S. Angle, and P. S. Bottomley (editors). Methods of soil analysis. Part 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, Wisconsin.

Iles, J., J. Kelleway, T. Kobayashi, D. Mazumder, L. Knowles, D. Priddel, and N. Saintilan. 2010. Grazing kangaroos act as local recyclers of energy on semiarid floodplains. Australian Journal of Zoology 58:145–149.

Kingsford, R. T., and R. F. Thomas. 2004. Destruction of wetlands and waterbird populations by dams and irrigation on the Murrumbidgee River in arid Australia. Environmental Management 34:383–396.

Lefroy, R. D. B., G. J. Blair, and W. M. Strong. 1993. Changes in soil organic-matter with cropping as measured by organic carbon fractions and ^{13}C natural isotope abundance. Plant and Soil 155: 399–402.

MacGregor, J. S. 2006. Effects of floodplain inundation and grazing on floodplain soil carbon dynamics and microbial community structure. PhD Thesis, Charles Stuart University, Albury, Australia.

Marx, M. C., M. Wood, and S. C. Jarvis. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry 33:1633–1640.

Morton, S. R., D. M. Stafford Smith, C. R. Dickman, D. L. Dunkerley, M. H. Friedel, R. R. J. McAllister, J. R. W. Reid, D. A. Roshier, M. A. Smith, F. J. Walsh, G. M. Wardle, I. W. Watson, and M. Westoby. 2011. A fresh framework for the ecology of arid Australia. Journal of Arid Environments 75:313–329.

Newman, S., J. B. Grace, and J. W. Koebel. 1997. Effects of nutrients and hydroperiod on *Typha*, *Cladium*, and *Eleocharis*: implications for Everglades restoration. Ecological Applications 7: 774–783.

Noy-Meir, I. 1973. Desert ecosystems: environment and producers. Annual Review of Ecology and Systematics 4:25–51.

O'Connell, M., D. S. Baldwin, A. I. Robertson, and G. Rees. 2000. Release and bioavailability of dissolved organic matter from floodplain litter: influence of origin and oxygen levels. Freshwater Biology 45:333–342.

Ogle, K., and J. F. Reynolds. 2004. Plant responses to precipitation in desert ecosystems: integrating functional types, pulses, thresholds, and delays. Oecologia (Berlin) 141:282–294.

Page, K., A. Read, P. Frazier, and N. Mount. 2005. The effect of altered flow regime on the frequency and duration of bankfull discharge: Murrumbidgee River, Australia. River Research and Applications 21:567–578.

Patrick, W. H., and K. R. Reddy. 1976. Fate of fertilizer nitrogen in a flooded rice soil. Soil Science Society of America Journal 40: 678–681.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Development Team. 2013. nlme: linear and nonlinear mixed effects models. R package version 3.1-109. R Project for Statistical Computing, Vienna, Austria.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, New York.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149.

Rayment, G. E., and F. R. Higginson. 1992. Australian laboratory handbook of soil and water chemical methods. Inkata Press, Melbourne, Australia.

Sharma, P., T. Asaeda, M. Kalibbala, and T. Fujino. 2008. Morphology, growth and carbohydrate storage of the plant *Typha angustifolia* at different water depths. Chemistry and Ecology 24: 133–145.

Silva, T. S. F., M. P. F. Costa, and J. M. Melack. 2009. Annual net primary production of macrophytes in the eastern Amazonian floodplain. Wetlands 29:747–758.

Spivak, A. C., M. J. Vanni, and E. M. Mette. 2011. Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales? Freshwater Biology 56: 279–291.

Stemmer, M., M. H. Gerzabek, and E. Kandeler. 1998. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. Soil Biology and Biochemistry 30:9–17.

Stevenson, F. J. 1994. Humus chemistry: genesis, composition, reactions. John Wiley and Sons, New York.

Tockner, K., M. Pusch, D. Borchardt, and M. S. Lorang. 2010. Multiple stressors in coupled river–floodplain ecosystems. Freshwater Biology 55:135–151.

Unger, I. M., A. C. Kennedy, and R. M. Muzika. 2009. Flooding effects on soil microbial communities. Applied Soil Ecology 42: 1–8.

Valett, H. M., M. A. Baker, J. A. Morrice, C. S. Crawford, M. C. Molles, C. N. Dahm, D. L. Moyer, J. R. Thibault, and L. M. Ellis. 2005. Biogeochemical and metabolic responses to the flood pulse in a semiarid floodplain. Ecology 86:220–234.

Wang, W. J., R. C. Dalal, P. W. Moody, and C. J. Smith. 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biology and Biochemistry 35: 273–284.

Wilson, J. S., D. S. Baldwin, G. N. Rees, and B. P. Wilson. 2011. The effects of short-term inundation on carbon dynamics, microbial community structure and microbial activity in floodplain soil. River Research and Applications 27:213–225.