Ecology Letters, (2004) 7: 829–836 doi: 10.1111/j.1461-0248.2004.00638.x

REPORT

Protein accumulation and distribution in floodplain soils and river foam

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Abstract

Many processes contribute to nutrient transfer from terrestrial to aquatic systems, but in most cases the contribution of particular organisms is unknown. In this study, we explore how a Bradford-reactive soil protein (BRSP) produced by arbuscular mycorrhizal fungi may provide nutrients to river ecosystems. Along a floodplain in Montana, we extracted BRSP from soils and related the protein concentrations to the age of soil surfaces. We identified BRSP in surface soils, as well as to a depth of 1.4 m, and found that the protein accumulates through time. We also detected BRSP in foam from five rivers in the western United States. Experiments were conducted that demonstrate that the protein may be leached or washed from soils and become a constituent of foam when mixed into turbulent water. We propose that terrestrially derived soil protein may enter rivers via erosion and leaching and serve as a nutrient source for aquatic organisms.

Keywords

Arbuscular mycorrhiza, Bradford-reactive soil protein, floodplain, foam, glomalin, *Populus*, soil carbon, soil structure.

Ecology Letters (2004) 7: 829–836

INTRODUCTION

The flow of nutrients between terrestrial and aquatic habitats in riparian zones is facilitated by flooding (Junk *et al.* 1989), percolation of water through sediments and soil (Wondzell & Swanson 1996), and groundwater flow (Brunke & Gonser 1997; Hill 2000). It is known that allochthonous sources of organic matter support high levels of benthic and hyporheic metabolism in rivers (Pusch *et al.* 1998; Tockner *et al.* 1999; Baker *et al.* 2000), but little is known about the sources or the amount of organic matter that is transported between floodplain soils and rivers. In terrestrial ecosystems the movement of nutrients between above- and below-ground environments is often mediated by mycorrhizal fungi that exchange phosphorus and nitrogen for plant carbon (Smith & Read 1997). Arbuscular mycorrhizal fungi associate with *c.* 80% of plant taxa (Allen 1991) and may utilize up to 45% of the carbon fixed by photosynthesis (Grayston *et al.* 1997) and subsequently store a fraction of this carbon in soil (Rillig *et al.* 2001; Lovelock *et al.* 2004a). The central role that arbuscular mycorrhizal fungi play in the transfer of carbon between plants and the soil ecosystem led us to investigate their role in transferring nutrients from floodplain soils to rivers.

Arbuscular mycorrhizal fungi (AMF) contribute to nutrient storage in soil directly via the formation of mycelial networks, as well as indirectly by affecting the structure of soil (Miller & Jastrow 2000). AMF produce extensive hyphae in soil that may exceed 100 m of hyphal length per gram of soil (Miller *et al.* 1995). Hyphae, along with plant roots, enmesh soil particles and contribute to the binding of soil particles (Tisdall *et al.* 1997; Jastrow *et al.* 1998). The contribution of AMF to soil structure, defined as the size and arrangements of particles and pores in soil (Hartge & Stewart 1995), is important because the formation of stable aggregates increases the storage of organic matter in the soil (Jastrow *et al.* 1996; Jastrow 1996), improves water infiltration and soil porosity (Paul & Clark 1989), and provides resistance to erosion (Sumner 2000). AMF also produce biochemical compounds that are important in soil aggregation. Of particular interest is the production of glomalin (Wright *et al.* 1996), a putative glycoprotein of as yet unknown biochemical structure that is operationally defined as Bradford-reactive soil protein (BRSP) (Rillig 2004). BRSP supplies nutrients to soil through the structure of the molecule, which in tropical soils has been measured as *c.* 37% C and 4% N, representing 3 and 5% of soil carbon and nitrogen pools, respectively (Lovelock *et al.* 2004a). BRSP also enhances soil nutrient storage via its contribution to soil

aggregate stabilization (Wright & Upadhyaya 1998; Rillig *et al.* 2002; Rillig 2004).

The objectives of this study were to characterize the distribution and accumulation of Bradford-reactive soil protein in floodplain soil and to explore potential contributions of BRSP to floodplain ecosystem processes. Because BRSP has been identified in a range of ecosystems (Wright & Upadhyaya 1998; Rillig *et al.* 2001; Lutgen *et al.* 2003) and a number of plant species on floodplains and in wetlands associate with arbuscular mycorrhizal fungi [i.e. various herbaceous understory species, *Populus* and *Salix* species (Vozzo & Hacskaylo 1974; Allen 1991; Smith & Read 1997; Bauer *et al.* 2003)], we hypothesized that BRSP would be present in floodplain soils. Soils are transported through the floodplain during floods, so we expected that freshly deposited sediment would have low concentrations of protein, but that after sediment deposition, protein would accumulate through time due to its known recalcitrance (Wright & Upadhyaya 1996; Rillig *et al.* 2003). We also hypothesized that BRSP could enter river flow via soil flushing that promotes the percolation of materials through the soil profile, by erosion, and through seasonal change in water table elevation that saturates the root zone where BRSP production occurs. Because BRSP may have hydrophobic properties [as it tends to accumulate at the air–water interface of flooded AMF sand cultures (Rillig, unpubl. observ.), and since it attaches to horticultural mesh inserted into AMF pot cultures (Wright *et al.* 1996)], we expected that if it did enter river water, it would float to the surface and become part of foam. Our results provide the first evidence of the occurrence of BRSP in floodplain soil and suggest mechanisms by which BRSP may move between terrestrial and aquatic environments.

MATERIALS AND METHODS

Study site

This study was conducted on the Nyack Floodplain of the Middle Fork of the Flathead River in northwestern Montana, USA (48°29′ N, 114°00′ W). The Middle Fork of the Flathead River is a fifth order freely-flowing river that flows along the southern boundary of Glacier National Park. The floodplain is 9 km long and 3 km wide and is delineated at the up- and downstream ends by bedrock constriction points. Between the constriction points, the river forms a braided channel with high hydrologic connectivity between surface and subsurface flow (Stanford & Ward 1993; Poole *et al.* 2002). In general, the upstream region of the floodplain is a losing reach where surface water enters the alluvial aquifer (downwells), whereas the downstream region is a gaining reach where groundwater enters surface flow (upwells). The gaining reach tends to have finer-textured soils and higher water table elevations (Harner & Stanford 2003) and may receive nutrient subsidies from hyporheic flow (Stanford & Ward 1993) compared with the losing reach. Cottonwood forests (*Populus trichocarpa*) and shrub communities of *Salix* species, *Alnus incana*, *Cornus stolonifera*, and *Lonicera involucrate*, as well as an herbaceous understory dominate frequently inundated reaches of the floodplain (Mouw & Alaback 2003). During a previous study Harner & Stanford (2003) established study plots in cottonwood forests along the Nyack Floodplain; half of the plots were located in the losing reach and half in the gaining reach. The ages of cottonwood trees were determined in the summer of 2000 by extracting increment cores and counting radial growth rings.

Soil collection

To document BRSP in floodplain soils through time in relation to age of soils and position on the floodplain, we collected soil samples from the previously established cottonwood plots (Harner & Stanford 2003) on the floodplain. We used the mean age of cottonwood trees within each plot to estimate the age of surfaces on Nyack Floodplain because cottonwoods tend to grow in even-aged stands, with the age of the stand corresponding to flood events that made germination possible (Sigafoos 1964; Everitt 1968). At some sites, tree age may over-estimate the age of sediments because floods may have deposited sediment after the cottonwoods established. In August 2000, soil pits were dug in the center of each of the cottonwood plots ($n = 31$) until gravel or cobble was reached. Soil was collected in each pit at a depth ranging from 0–20 cm, except for one sample that was collected at 50 cm. In 10 of the pits, soil was collected vertically through the profile from the middle of each soil layer. The maximum depth sampled was 1.4 m. Soil was air-dried and stored at room temperature for 5 months prior to analyses.

Extraction of protein

Protein extractions from soil were carried out as described by Wright & Upadhyaya (1998). Easily extractable Bradford-reactive soil protein (EE-BRSP) was extracted with 20 mM citrate, pH 7.0 at 121 °C for 30 min. Total BRSP was extracted with 50 mM citrate, pH 8.0 at 121 °C in rounds of 60 min each. For the sequential extractions, the supernatant was removed by centrifugation at $5000 \times \boldsymbol{g}$ for 20 min. Extraction of a sample continued until the supernatant showed none of the reddish-brown color typical of BRSP. After extraction cycles were completed, samples were centrifuged at $10\,000 \times \boldsymbol{g}$ to remove soil particles, and the protein concentration in the supernatant was determined using a Bradford assay (Wright & Upadhyaya 1998). In this

study, glomalin was not quantified using an indirect enzyme-linked immunosorbent assay (ELISA) (Wright & Upadhyaya 1996) because other research has demonstrated that BRSP and immunoreactive soil protein (IRSP) are highly correlated in the soils from this floodplain [EE-BRSP with EE-IRSP: $r^2 = 0.787$, $P = 0.0012$; $y = 0.022 + 0.887X$; total BRSP with total IRSP: $r^2 = 0.6437$, $P = 0.0017$; $y = -0.12 + 0.802X$; (Piotrowski and Rillig, unpubl.)]. This correlation between BRSP and IRSP links the origin of the substances from the floodplain detected by the Bradford assay more strongly to arbuscular mycorrhizal fungi (Wright *et al.* 1996; Wright & Upadhyaya 1996).

Soil aggregation and hyphal lengths

For characterization of aggregate water stability, macro-aggregates of 1–2 mm diameter were measured because responses of this aggregate size class have been correlated with BRSP concentrations in previous studies (Wright & Upadhyaya 1998). Replicate 4 g samples of soil were moistened by capillary action for 10 min. Water stability of aggregates was measured with the wet-sieving method described in Kemper & Rosenau (1986). The initial and final weights of aggregates were corrected for the weight of coarse particles (>0.25 mm). Aggregate stability was calculated as the mass of aggregated soil remaining after wet sieving as a percent of the total mass of soil. Because some studies have shown hyphal lengths to be correlated with the percentage of water stable aggregates, hyphae were extracted from a 4 g soil sub-sample by an aqueous extraction and membrane filter technique modified after Jakobsen *et al.* (1992), as described in Rillig *et al.* (1999). Hyphal lengths were measured from air-dried soils from 12 of the study plots in the losing reach.

Characterization of foam and protein transport to water

To determine if Bradford-reactive soil protein is present in river foam, foam was sampled from the surface of river water from one location on the Gila, Jemez, and Rio Grande in New Mexico and the Bitterroot and the Middle Fork of the Flathead River in Montana. Foam was collected from the river where it naturally accumulates in backwater eddies. At one site on the Middle Fork of the Flathead River a soil pit was dug to the point where the pit intersected the water table and foam that accumulated on the water table was sampled. The foam was immediately frozen and was stored for up to 6 months prior to analysis. The foam samples were freeze-dried to yield dry residues; protein content of the foam was determined using a Bradford assay (Wright & Upadhyaya 1998).

To determine if BRSP could enter water from soils and then constitute foam, two laboratory experiments were conducted to obtain BRSP in solution via leaching and washing soil. For both experiments the sub-samples of soils collected from the A horizons on the floodplain were homogenized. For the leaching experiment, 30 g of the homogenized soil was placed in a 50 mL centrifuge tube that had the bottom cut off. Mesh (38 μm) was placed over the cut end. Water was added to the soil in 10 mL increments up to 110 mL, and the water that flowed through the mesh was captured. The captured water was autoclaved and analyzed by the Bradford assay. For the washing experiment, 50 g of soil and 100 mL of water were combined in a 250 mL round-bottom glass flask. The flasks were placed on a shaker table and shaken overnight. Foam that collected on the sides of the flask was scraped off, dried, weighed, and extracted with the citrate buffer, autoclaved, and then analyzed by the Bradford assay (Wright & Upadhyaya 1998).

To estimate BRSP contributions to soil carbon and nitrogen on the floodplain and potentially to nutrient flux from the terrestrial to the aquatic environment, we related the mean weight of BRSP per gram of soil to an estimate of the volume of soil eroded into the river each year. We calculated the mean concentration of BRSP in soils from Nyack floodplain and related the concentration of BRSP to the bulk density of the soils (1.19 g cm^{-3}; Harner *unpubl. data*). To estimate the volume of sediment eroding from channel banks to the river, we used an approximation proposed by Everitt (1968) of the volume of sediment eroded from channel banks on the Little Missouri River of western North Dakota (31 671 m^3 km^{-1}). We calculated the mass of BRSP potentially entering river flow from channel erosion each year as a function of the volume of soil eroded, the density of the soil, and the concentration of BRSP in soil. We then estimated carbon and nitrogen in BRSP that could potentially flux from the floodplain into the river each year. Because BRSP is estimated to be between 28 and 43% carbon (S. Wright, pers. comm.) and 3–5% N (Lovelock *et al.* 2004a), we used values of 30 and 4% as approximations of C and N content of glomalin, respectively.

Data analysis

We analyzed the combined data from all plots, as well as the data from plots in the losing vs. the gaining reach of the floodplain. Total BRSP was correlated with EE-BRSP using Spearman rank correlations. To describe the accumulation of total BRSP through time, logistic regression was used to determine the shape of the functional response followed by nonlinear least-squares regression of total BRSP vs. age. An analysis of covariance (ANCOVA) was conducted on log-transformed data to compare BRSP between the losing and the gaining reach while controlling for age. To estimate turnover rates of protein, a linear regression of ln (protein

concentration) as a function of cottonwood stand age was conducted, and the inverse of the slope was taken as an estimate of turnover time. We examined the relationships between the percentage of water stable aggregates (WASs) and BRSP using linear least-squares regression. Spearman rank correlations were used to relate hyphal lengths to BRSP and the percentage of water stable aggregates. The statistics program SPSS (version 7.5 for Windows) (SPSS 1997) was used. The presence of outlying data points were identified using box plots. If a point was located more than three interquartile ranges away from the box, the value was removed from the analysis. Correlations and differences were considered significant at $P < 0.05$.

RESULTS

On the Nyack Floodplain of the Middle Fork of the Flathead River in western Montana, USA, Bradford-reactive soil protein was present in all surface soils that were sampled. BRSP ranged from 0.42–8.67 mg protein g^{-1} of soil [mean: 3.38 (0.43 SE) mg g^{-1}, $n = 30$]; EE-BRSPP ranged from 0.23–2.49 mg protein g^{-1} of soil [mean 0.96 (0.10 SE) mg g^{-1}, $n = 30$]. One plot in the losing reach was an outlier and had a concentration of total BRSP of 10.24 mg protein g^{-1} of soil and was removed from all analyses. The concentration of total BRSP was correlated with EE-BRSP in the surface soils (Spearman's *rho* = 0.886, $P < 0.001$, $n = 30$). Total BRSP was strongly correlated with the age of the site (Fig. 1). Higher concentrations of BRSP were found in soils collected from the gaining reach compared to the losing reach of the floodplain ($F = 12.38$, $P = 0.001$, d.f. = 1; Fig. 1).

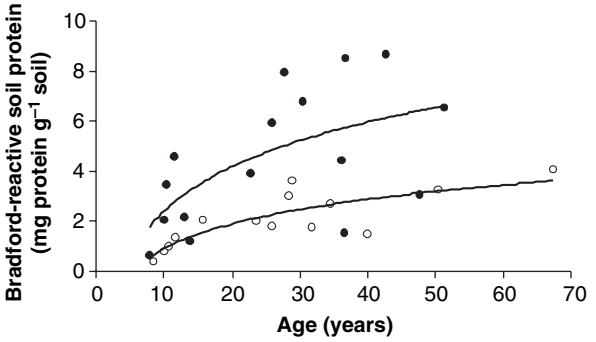

Figure 1 The relationship between Bradford-reactive soil protein (y) and site age (x) in the gaining reach (closed circles) and losing reach (open circles). The regression for the gaining reach: $y = 2.57 \ln(x) - 3.51$ ($r^2 = 0.37$, $P = 0.013$, $n = 16$). The regression for the losing reach: $y = 1.41 \ln(x) - 2.32$ ($r^2 = 0.68$, $P < 0.001$, $n = 14$). The regression for all plots combined (regression line not shown): $y = 1.97 \ln(x) - 2.78$ ($r^2 = 0.26$, $P = 0.003$, $n = 30$).

The concentration of BRSP was correlated with the percentage of WASs in these floodplain soils (Spearman's *rho* = 0.709, $P < 0.001$, $n = 30$; Fig. 2a). EE-BRSP also was correlated with the percentage of WSAs (*Speraman's rho* = 0.580, $P = 0.001$, $n = 30$). The relationship between BRSP and $WSA_{1-2\ mm}$ for all plots combined is a decreasing function and shows that at a threshold of $WSA_{1-2\ mm}$ between 15 and 20%, an increase in BRSP has little effect on the stabilization on soil particles. However, BRSP and $WSA_{1-2\ mm}$ are related linearly when isolated by reach (Fig. 2b). In the plots in the losing reach where hyphal lengths were measured, a relationship between the aggregation of soil particles and hyphal lengths was not detected. Hyphal lengths ranged from 3.4 to 41.3 cm g^{-1} of soil with a mean of 18.2 [4.0 SE] cm g^{-1}. The length of hyphae was not correlated with total BRSP (Spearman's *rho* = 0.375, $P = 0.229$, $n = 12$), nor with WSAs (Spearman's *rho* = 0.399, $P = 0.198$, $n = 12$), but total BRSP was positively

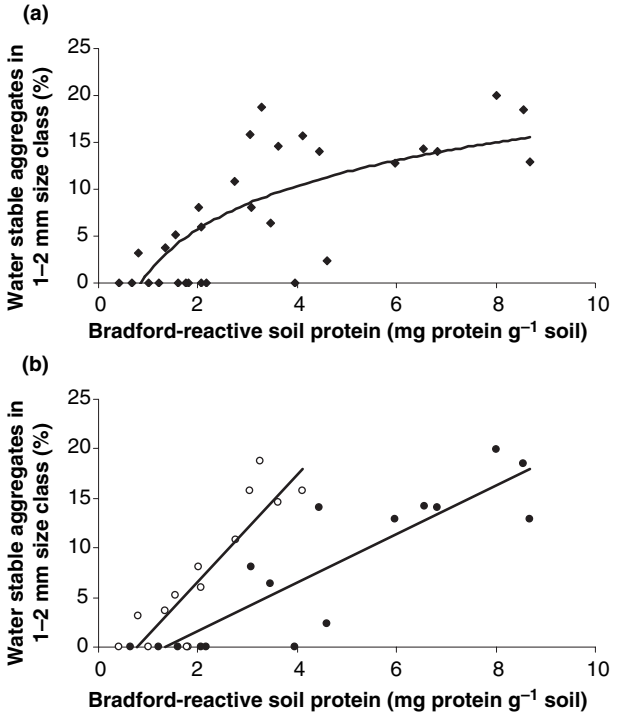

Figure 2 (a) Water stable aggregates (WSAs) in the 1–2 mm size class (y) as a function of Bradford-reactive soil protein (x) for all plots. The regression for all plots combined: $y = 6.68 \ln(x) + 1.10$ ($r^2 = 0.546$, $P < 0.001$, $n = 30$). (b) WASs in the 1–2 mm size class (y) as a function of Bradford-reactive soil protein (x) in the losing reach (open circles) and the gaining reach (closed circles). The regression for the losing reach: $y = 5.43x - 4.21$ ($r^2 = 0.79$, $P < 0.001$, $n = 14$). The regression for the gaining reach: $y = 2.46x - 3.33$ ($r^2 = 0.78$, $P < 0.001$, $n = 16$).

correlated with WSA (Spearman's *rho* = 0.789, $P = 0.002$, $n = 12$).

Along with detecting BRSP in surface soils, we identified BRSP vertically through the soil profile in all plots and at all depths measured, in river foam, and in foam and water extracts from laboratory experiments. In the floodplain soils there was no clear trend for a decrease in BRSP with depth, or for a difference in concentration of BRSP at depth between the losing and gaining reaches (Fig. 3). One of these soil pits intersected the water table, and when the pit filled with water, a layer of foam accumulated on the water surface. Analysis of this foam revealed that the foam contained Bradford-reactive protein that had a concentration of 1.2 mg protein g^{-1} of freeze-dried foam. Bradford-reactive protein also was identified in all of the foam samples that were collected from rivers (Table 1). The foam measurements are qualitative because only small amounts of foam were available after freeze-drying (<0.01 g per sample), and the precision of the measurement was likely negatively affected by the small amount of sample material. In the laboratory leaching experiment where water was poured through samples, BRSP was recovered in the water extract. The mean concentration of BRSP in the water extract was 0.019 (0.003 SE) mg protein g^{-1} of soil ($n = 4$), and demonstrates that a fraction of the protein (*c.* 0.56%) moved from the soil into the leachate. A higher fraction of protein (*c.* 32%) was removed from the soil when the soils were washed via shaking overnight [1.067 (0.04 SE) mg protein g^{-1} of soil, $n = 3$).

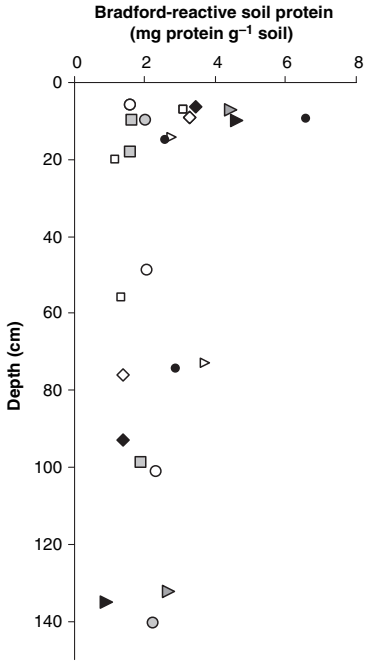

Figure 3 Concentration of Bradford-reactive soil protein at depth. Each symbol corresponds to an individual soil profile ($n = 10$). Soil profiles from the losing reach ($n = 4$) are shown as open symbols; soil profiles from the gaining reach ($n = 6$) are shown as closed symbols.

Table 1 Bradford-reactive protein extracted from river foam

River	River foam (mg protein g^{-1} of foam)
MF Flathead River	8.17
Bitteroot River	9.66
Jemez River	0.67
Rio Grande	0.10
Gila River	1.49

The results of the laboratory experiments demonstrate that the protein can be physically removed from the soil and transported to water, such as might occur following the erosion of soils during floods. We estimate the potential input of BRSP to the river from erosion to be 1.27×10^5 kg km^{-1} of river length. If BRSP is *c.* 30% carbon and 4% nitrogen, then *c.* 3.80×10^4 kg C and 5.00×10^3 kg N may enter the river every year over each kilometer of river due to channel erosion and the washing of soil into river flow.

DISCUSSION

This is the first study to show that BRSP, a glycoprotein of soil and arbuscular mycorrhizal fungal origin, is present in alluvial soils, as well as in river foam. On the Nyack Floodplain of the Middle Fork of the Flathead River in Montana we found BRSP in all surface soils and soil profiles that were sampled. The concentrations of total BRSP in the floodplain soils were in the range of concentrations of the protein in soils from the mid-Atlantic area of the United States (Wright & Upadhyaya 1996), California grasslands (Rillig *et al.* 2002), croplands in the central Great Plains (Wright & Anderson 2000), and in a tropical rain forest in Costa Rica (Lovelock *et al.* 2004a). In addition to identifying Bradford-reactive protein in soil, we detected Bradford-reactive protein in river foam that was collected from five western North American rivers and demonstrated in laboratory experiments that the protein can be leached and washed from soil.

We were able to characterize the accumulation of BRSP over a fluvial deposition chronosequence (Fig. 1) by relating the concentration of BRSP in the soil to the age of the soil, which we estimated using the age of cottonwood trees that established following floods (Sigafoos 1964; Everitt 1968; Karrenberg *et al.* 2002). The relationship between BRSP and site age suggests that the protein accumulates through time

and has a turnover time of about 35 years. The accumulation of BRSP in the floodplain soils is consistent with findings that have shown BRSP to be persistent in soils (Wright & Upadhyaya 1996; Rillig *et al.* 2001). The rate of increase in BRSP concentration on the floodplain tended to decrease with time, which may have resulted from turnover of labile pools of the protein (Lutgen *et al.* 2003).

The increase in BRSP in the soil through time is important because the protein may contribute to the stabilization of soil particles. In this study we observed that over time and as concentrations of BRSP increased the percentage of soil with aggregates in the 1–2 mm size class increased (Fig. 2a,b). These results are similar to the findings of Wright & Upadhyaya (1998) that show BRSP to be highly correlated with aggregate stability. We also found that soils in the losing reach have more aggregate stability than soils in the gaining reach for a given concentration of BRSP (Fig. 2b), which may be related to deposition of heavier (possibly more aggregated particles) in the upstream portion of the floodplain. In contrast to some studies that have shown WSA_{1-2mm} to be correlated with hyphal lengths in soil (e.g. Jastrow *et al.* 1998), we did not detect a relationship between the aggregation of soil particles and hyphal lengths. Hyphal lengths were small in these soils, ranging from 3.4–41.3 cm g^{-1} of soil, compared with other systems that have hyphal lengths in the order of tens of meters per gram of soil (Miller *et al.* 1995).

Concentrations of BRSP were greater in the gaining reach of the floodplain compared to the losing reach, but at present, we do not have an explanation for this difference. Differences in BRSP production could explain differences in glomalin concentrations in soils among the losing and gaining reaches. Alternatively, transport of BRSP from the losing reach downstream to the gaining reach could result in deposition of BRSP during floods and contribute to higher concentrations of the protein in the gaining reach. Previous studies have shown concentrations of BRSP to be positively correlated with the availability of soil carbon and nitrogen (Rillig *et al.* 2003). In tropical soils, Lovelock *et al.* (2004a) found that less fertile soils had higher stocks of glomalin, but concentrations of putatively younger immunofluorescent glomalin were lower in less fertile soils compared to more fertile soils. They suggested that glomalin production is lower in less fertile soils and higher in more fertile soils and subsequently confirmed this in direct assays of glomalin accumulation from in-growth cores (Lovelock *et al.* 2004b).

In our study, the greater concentrations of BRSP in the gaining reach could be a response to localized nutrient subsidies in soils in the gaining region of the floodplain. Nutrient subsidies to plants and soil potentially come from groundwater that may be enriched in nitrogen and phosphorus in this region of the floodplain (Stanford & Ward 1993). These nutrient subsidies also may result from increased mineralization of nutrients during periods of inundation, which may occur more frequently or for longer durations in the gaining reach due to the accumulation of river water above the downstream constriction point on the river. In addition, soil texture, which tends to be finer over a greater depth in this region (Harner & Stanford 2003), may affect plant species composition and soil nutrient content and may favor the production or persistence of BRSP in the gaining reach of the floodplain. The contribution and interaction of soil nutrients, soil texture, soil moisture, and plant community structure to BRSP production and accumulation require additional study.

BRSP is present in soils that are hydrologically connected with the river and, as a result, has the potential to enter river flow via leaching and erosion. The occurrence of BRSP at depth in the soil profiles suggests that protein may move vertically in the soil profile or be deposited at depth because the roots of cottonwood trees extend several meters below the soil surface. On this floodplain peak river discharge occurs from May through June following snowmelt, during which time channels migrate across the floodplain, soil is eroded from the riverbanks, and the water table rises through the soil profile. During these times, soils containing BRSP may enter the river and subsequently be washed during transport, thus releasing BRSP from the soil matrix. Because our laboratory experiments demonstrated that *c.* 30% of BRSP was released from the soil via washing, we expect erosion of soils and physical disturbance of soil particles to be an important mechanism for release of the protein into river water. BRSP also may be leached from the soil and transported to the river via groundwater as the water table rises throughout the soil profile where BRSP occurs. This leaching of BRSP through the soil and into groundwater may over-ride the contributions from erosion because the spatial extent of interaction zones between floodplain soils and the hyporheic zone, which may extend up to several kilometers from the river channel (Stanford & Ward 1988), are so much larger than the channel lengths that are subject to erosion.

Along with affecting aggregate stability, BRSP might act as a nutrient source for aquatic food webs by contributing carbon and nitrogen from terrestrial sources to water via erosion and leaching of floodplain soils that contain the protein. Hyporheic zones, the subsurface regions where surface and groundwater mix, provide a critical link for nutrient exchange between terrestrial and aquatic environments (Stanford & Ward 1988, 1993; Wondzell & Swanson 1996; Brunke & Gonser 1997; Hill 2000). Hyporheic zones host diverse and productive interstitial microbial communities (Ellis *et al.* 1998; Craft *et al.* 2002) and invertebrate communities with groundwater affinities (Stanford & Ward 1988). However, sources of carbon to support hyporheic metabolism appear to be limiting or not accounted for in

many alluvial river ecosystems (Ellis *et al.* 1998; Craft *et al.* 2002). Carbon and nutrient inputs from the soil, such as from BRSP, may enter groundwater and support a portion of this hyporheic metabolism.

This study provides an example of a previously unknown link between terrestrial and aquatic systems, a link for carbon and nitrogen transport from mycorrhizal fungi to river water. Three lines of evidence indicate that terrestrial AM fungi link terrestrial and aquatic systems through the secretion of BRSP. First, BRSP accumulates in river floodplain soils that are frequently saturated and are continuously recycled by the river. Second, BRSP, which has been shown to be downwardly mobile in soil, is found in the floodplain aquifer and in soils that lie within the seasonal boundary of the aquatic system. Third, BRSP is present in river foam, which suggests that the protein may move from the soil to rivers. The findings presented here document an example of specific linkage between a river and its floodplain that would be severed by measures that constrain channel migration and disconnect a river from a surrounding aquifer. This work also illustrates another example of the wide-spread occurrence of BRSP in an ecosystem and highlights potential contributions of the protein to river ecosystem processes, including structuring of soil, storage of nutrients, and nutrient transfer.

ACKNOWLEDGEMENTS

We thank Jake Chaffin, Rebecca Lawrence, and Jason Mouw for field assistance and the Dalimata family for access to the study site. Jeff Piotrowski provided the immunoreactivity-Bradford correlations. We thank Robert Sinsabaugh and two referees for their comments on the manuscript. This research was supported by a NSF Integrative Graduate Education and Research Training Award (DGE-9972810), a NSF Biocomplexity Grant (DEB-0083422), a NSF award (DEB-0083884) to the Flathead Lake Biological Station of the University of Montana, and by a NSF Award (DEB-0128953) to MCR.

REFERENCES

Allen, M.F. (1991). *The Ecology of Mycorrhizae*. Cambridge University Press, Cambridge, UK.

Baker, M.A., Valett, H.M. & Dahm, C.N. (2000). Organic carbon supply and metabolism in a shallow groundwater ecosystem. *Ecology*, 81, 3133–3148.

Bauer, C.R., Kellogg, C.H., Bridgham, S.D. & Lamberti, G.A. (2003). Mycorrhizal colonization across hydrologic gradients in restored and reference freshwater wetlands. *Wetlands*, 23, 961–968.

Brunke, M. & Gonser, T. (1997). The ecological significance of exchange processes between rivers and groundwater. *Freshwater Biol.*, 37, 1–33.

Craft, J.A., Stanford, J.A. & Pusch, M. (2002). Microbial respiration within a floodplain aquifer of a large gravel-bed river. *Freshwater Biol.*, 47, 251–261.

Ellis, B.K., Stanford, J.A. & Ward, J.V. (1998). Microbial assemblages and production in alluvial aquifers of the Flathead River, Montana, USA. *J. N. Am. Benthol. Soc.*, 17, 382–402.

Everitt, B.L. (1968). Use of the cottonwood in an investigation of the recent history of a flood plain. *Am. J. Sci.*, 266, 417–439.

Grayston, S., Vaughan, D. & Jones, D. (1997). Rhizosphere carbon flow in trees in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Appl. Soil Ecol.*, 5, 29–56.

Harner, M.J. & Stanford, J.A. (2003). Differences in cottonwood growth between a losing and a gaining reach of an alluvial floodplain. *Ecology*, 84, 1453–1458.

Hartge, K.H. & Stewart, B.A. (1995). Soil structure: its development and function. *Advanced Soil Science*. CRC, Lewis Publishers, Boca Raton, FL, USA.

Hill, A.R. (2000). Stream chemistry and riparian zones. In: *Streams and Ground Waters* (ed. Jones, J.B. & Mulholland, P.J.). Academic Press, San Diego, CA, USA, pp. 83–110.

Jakobsen, I., Abbott, L.K. & Robson, A.D. (1992). External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytol.*, 120, 371–380.

Jastrow, J.D. (1996). Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. *Soil Biol. Biochem.*, 28, 665–676.

Jastrow, J.D., Boutton, T.W. & Miller, R.M. (1996). Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. *Soil Sci. Soc. Am. J.*, 60, 801–807.

Jastrow, J.D., Miller, R.M. & Lussenhop, J. (1998). Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. *Soil Biol. Biochem.*, 30, 905–916.

Junk, W.J., Bayley, P.B. & Sparks, R.E. (1989). The flood pulse concept in river-floodplain systems. In: *Proceedings of the International Large River Symposium*. (ed. Dodge, D.P.). Canadian Special Publication in Fisheries and Aquatic Science. pp. 110–127.

Karrenberg, S., Edwards, P.J. & Kollmann, J. (2002). The life history of Salicaceae living in the active zone of floodplains. *Freshwater Biol.*, 47, 733–748.

Kemper, W.D. & Rosenau, R.C. (1986). Aggregate stability and size distribution. In: *Methods of Soil Analysis (Part 1)*. (ed. Klute, A.). American Society of Agronomy, Madison, WI, pp. 442–452.

Lovelock, C.E., Wright, S.F., Clark, D.A. & Ruess, R.W. (2004a). Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *J. Ecol.*, 94, 278–287.

Lovelock, C.E., Wright, S.F. & Nichols, K.A. (2004b). Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil. *Soil Biol. Biochem.*, 36, 1009–1012.

Lutgen, E.R, Muir-Clairmont, D., Graham, J. & Rillig, M.C. (2003). Seasonality of arbuscular mycorrhizal hyphae and glomalin in a western Montana grassland. *Plant Soil*, 257, 71–83.

Miller, R.M. & Jastrow, J.D. (2000). Mycorrhizal fungi influence soil structure. In: *Arbuscular Mycorrhizas: Physiology and Function* (eds Kapulnik, Y. & Douds, D.D.). Kluwer Academic Publishers, Dordrecht, pp. 3–18.

Miller, R.M., Reinhardt, D.R. & Jastrow, J.D. (1995). External hyphal production of vesicular–arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia*, 103, 17–23.

Mouw, J.E.B. & Alaback, P.B. (2003). Putting floodplain hyperdiversity in a regional context: an assessment of terrestrial-floodplain connectivity in a montane environment. *J. Biogeogr.*, 30, 87–103.

Paul, E.A. & Clark, F.E. (1989). *Soil Biology and Biochemistry*. Academic Press, San Diego, CA, USA.

Poole, G.C., Stanford, J.A., Frissell, C.A. & Running, S.W. (2002). Three-dimensional mapping of geomorphic controls on floodplain hydrology and connectivity from aerial photos. *Geomorphology*, 48, 329–347.

Pusch, M., Fiebig, D., Brettar, I., Eisenmann, H., Ellis, B.K., Kaplan, L.A. *et al.* (1998). The role of micro-organisms in the ecological connectivity of running waters. *Freshwater Biol.*, 40, 453–495.

Rillig, M. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Sci.*, in press.

Rillig, M.C., Field, C.B. & Allen, M.F. (1999). Soil biota responses to long-term atmospheric CO_2 enrichment in two California annual grasslands. *Oecologia*, 119, 572–577.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F. & Torn, M.S. (2001). Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil*, 233, 167–177.

Rillig, M.C., Wright, S.F. & Eviner, V.T. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant Soil*, 238, 325–333.

Rillig, M.C., Ramsey, P.W., Morris, S. & Paul, E.A. (2003). Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. *Plant Soil*, 253, 293–299.

Sigafoos, R.S. (1964). Botanical evidence of floods and flood-plain deposition. *US Geol. Surv. Prof. Pap.*, 485-A, 1–35.

Smith, S.E. & Read, D.J. (1997). *Mycorrhizal Symbiosis*. Academic Press, San Diego, CA, USA.

SPSS (1997). SPSS version 7.5 for Windows. SPSS, Chicago, IL, USA.

Stanford, J.A. & Ward, J.V. (1988). The hyporheic habitat of river ecosystems. *Nature*, 335, 64–66.

Stanford, J.A. & Ward, J.V. (1993). An ecosystem perspective on alluvial rivers-connectivity and the hyporheic corridor. *J. N. Am. Benthol. Soc.*, 12, 48–60.

Sumner, M.E. (2000). *Handbook of Soil Science*. CRC Press, Boca Raton, FL, USA.

Tisdall, J.M., Smith, S.E. & Rengasamy, P. (1997). Aggregation of soil by fungal hyphae. *Aust. J. Soil Res.*, 35, 55–60.

Tockner, K., Pennetzdorfer, D., Reiner, N., Schiemer, F. & Ward, J.V. (1999). Hydrological connectivity and the exchange of organic matter and nutrients in a dynamic river-floodplain system (Danube, Austria). *Freshwater Biology*, 41, 521–525.

Vozzo, J.A. & Hacskaylo, E. (1974). Endo- and ectomycorrhizal associations in five *Populus* species. *Bull. Torr. Bot. Club*, 101, 182–186.

Wondzell, S. & Swanson, F. (1996). Seasonal and storm dynamics of the hyporheic zone of a 4th-order mountain stream 2. Nitrogen cycling. *J. N. Am. Benthol. Soc.*, 15, 20–34.

Wright, S.F. & Anderson, R. (2000). Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. *Biol. Fertil. Soils*, 31, 249–253.

Wright, S.F. & Upadhyaya, A. (1996). Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci.*, 161, 575–586.

Wright, S.F. & Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil*, 198, 97–107.

Wright, S.F., Franke-Snyder, M., Morton, J. & Upadhyaya, A. (1996). Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant Soil*, 181, 193–203.

Editor, John Klironomos
Manuscript received 21 April 2004
First decision made 27 May 2004
Manuscript accepted 2 June 2004