

PLANT GENES LINK FORESTS AND STREAMS

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Abstract. Although it is understood that the composition of riparian trees can affect stream function through leaf litter fall, the potential effects of genetic variation within species are less understood. Using a naturally hybridizing cottonwood system, we examined the hypothesis that genetic differences among two parental species (*Populus fremontii* and *P. angustifolia*) and two groups of their hybrids (F₁ and backcrosses to *P. angustifolia*) would affect litter decomposition rates and the composition of the aquatic invertebrate community that colonizes leaves. Three major findings emerged: (1) parental and hybrid types differ in litter quality, (2) decomposition differs between two groups, a fast group (*P. fremontii* and F₁ hybrid), and a slow group (*P. angustifolia* and backcross hybrids), and (3) aquatic invertebrate communities colonizing *P. fremontii* litter differed significantly in composition from all other cross types, even though *P. fremontii* and the F₁ hybrid decomposed at similar rates. These findings are in agreement with terrestrial arthropod studies in the same cottonwood system. However, the effects are less pronounced aquatically than those observed in the adjacent terrestrial community, which supports a genetic diffusion hypothesis. Importantly, these findings argue that genetic interactions link terrestrial and aquatic communities and may have significant evolutionary and conservation implications.

Key words: aquatic decomposition; aquatic–terrestrial interaction; cottonwood hybridization; ecological genetics; genetic introgression; genetic variation; hybrids; leaf litter decomposition; macroinvertebrate communities; *Populus*.

INTRODUCTION

Streamside vegetation has the capacity to affect stream function through interactions mediated through leaf litter fall (Petersen and Cummins 1974, Cummins and Klug 1979, Cummins et al. 1989). Aquatic invertebrates and microbes that colonize fallen leaves can discriminate among leaf litter of different species (Webster and Benfield 1986, Petersen et al. 1989). We hypothesized that aquatic invertebrates could also discriminate among leaves at a genetic level due to differences in phytochemistry among and within cottonwood hybrids (e.g., Findlay and Jones 1990, Driebe and Whitham 2000, Schweitzer et al. 2004).

We chose cottonwoods (*Populus* sp.) for this study because of their prevalence in riparian areas throughout the western United States, where cottonwoods can comprise a dominant proportion of the biomass, as well

as the high levels of genetic diversity generated when cottonwoods naturally hybridize. In cottonwood riparian systems, natural hybrid zones are formed wherever two or more species overlap in distribution (Eckenswelder 1984), and molecular studies argue that hybrid speciation has been important in this genus (Smith and Sytsma 1990). Because natural hybridization is found in diverse taxa worldwide, and is thought to represent a major pathway in plant evolution (e.g., Stace 1987, Rieseberg et al. 1996), changes in the genetic structure of these systems could have community-wide consequences and apply to diverse systems.

If aquatic ecosystems respond to the genetic makeup of terrestrial inputs, then streamside restoration plantings of clonal replicates could sufficiently reduce the genetic variation of these inputs and disrupt ecosystem functioning in streams. To assess the effect of plant genes on aquatic communities, we made leaf litter decomposition bags of mixtures of Fremont cottonwood (*Populus fremontii* S. Wats.), narrowleaf cottonwood (*P. angustifolia* James), and naturally occurring F₁ and backcross cottonwood hybrids, which collectively are

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referred to as cross types (e.g., Wimp et al. 2005). This hybridizing system demonstrates unidirectional introgression, with the F_1 hybrid successively backcrossing with only the *P. angustifolia* parent (Keim et al. 1989, Martinsen et al. 2001). Using these litter bags we quantified decomposition rates and associated naturally coalescing macroinvertebrate communities.

Although aquatic communities have been shown to differentiate among leaf litters of different species (Webster and Benfield 1986), in general aquatic invertebrates are thought to be less specific to substrate quality than terrestrial arthropods due to a lack of host-specific feeding guilds and associative relationships (Cummins and Klug 1979, Vannote et al. 1980, Graça 2001; but see Ikeda and Nakasuji 2002). Therefore, we hypothesized a genetic diffusion hypothesis where we would expect a more diffuse relationship between plant genetics and aquatic invertebrates than plant genetics and terrestrial arthropods.

Recent studies in riparian cottonwood forests have shown that genetic variation among these same four cross types affects the composition of terrestrial arthropod communities (Floate and Whitham 1995, Wimp et al. 2004, 2005). We show for the first time that when leaf litter from these same cross types falls into a stream, aquatic invertebrates can also discriminate among them. Mechanisms for this discrimination may be leaf litter phytochemistry or rate of instream decomposition (Driebe and Whitham 2000). These results demonstrate genetic-level linkages between terrestrial and aquatic ecosystems and suggest that evolutionary processes in forests may cross ecosystem boundaries to affect community structure in streams.

MATERIALS AND METHODS

Site description

Oak Creek, Arizona, a tributary of the Verde River, is characterized by steep topography and sandstone/limestone bedrock. Riparian vegetation includes *P. fremontii*, *P. angustifolia*, and their naturally occurring hybrids, Arizona alder (*Alnus oblongifolia* Torr.), Arizona sycamore (*Platanus wrightii* S. Wats.), coyote willow (*Salix exigua* Nutt.), and Goodding's willow (*Salix gooddingii* Ball). The Oak Creek catchment has an area of 77 450 km² and ranges in elevation from 1371 to 2133 m. Oak Creek is an alkaline, well-aerated stream, dominated by bicarbonate, calcium, and magnesium.

Water quality parameters were measured throughout the study period (October 2001 to April 2002) using a Hydrolab minisonde 4a (Hydrolab-Hach Corporation, Loveland, Colorado, USA). Temperature, specific conductivity, dissolved oxygen, pH, total dissolved solids, and salinity were measured at each harvest date. Stream

temperature during leaf exposure ranged from 5.05° to 9.12°C, specific conductivity ranged from 297.8 to 299.9 μ S/cm, dissolved oxygen ranged from 40.3% to 62.0%, pH ranged from 7.72 to 8.92, total dissolved solids ranged from 0.1885 to 0.1982 mg/L, and salinity consistently measured 0.145‰.

Litter decomposition

To assess the ability of plant genes to affect aquatic communities, we used leaf litter decomposition bags of mixtures of known cottonwood genotypes and quantified their associated naturally coalescing macroinvertebrate communities. Cottonwood leaves were collected in mesh branch bags from trees planted in a common garden of known genetic composition (Ogden Nature Center, Ogden, Utah, USA). Naturally abscised leaves were collected from mesh branch bags frequently to minimize chances of leaching. Trees in the common garden were planted in 1991 and are now mature. The common garden removes environmental variation and insures that differences in tree physiology or leaf chemistry are likely due to genetic differences.

We collected litter from five to seven genotypes of each of the four cross types (*P. fremontii*, *P. angustifolia*, F_1 hybrid, backcross hybrid) in the fall of 2001. Air-dried leaf litter was ground for initial chemical analyses in a Wiley mill (3383L10 Thomas Scientific, Swedesboro, New Jersey, USA) to pass mesh size 40. We extracted condensed tannins from 25 to 50 mg subsamples with 70% acetone and 10 mmol/L ascorbic acid. We determined condensed tannin concentrations using the butanol-HCl method (Porter et al. 1986), with standards purified from *P. angustifolia* following the methods of Hagerman and Butler (1989). We quantified absorbance on a Spectramax-Plus 384 spectrophotometer (Molecular Devices, Sunnyvale, California, USA). We also determined total litter nitrogen and phosphorus by modified micro-Kjeldahl digestion (Parkinson and Allen 1975) and analysis on a Lachat AE Flow Injection Analyzer (Lachat Instruments, Loveland, Colorado, USA), using the salicylate and molybdate-ascorbic acid methods, respectively (Lachat Instruments 1992). Initial organic carbon content was determined by combusting in a muffle furnace (Barnstead International 47000, Dubuque, Iowa, USA) at 500°C for 1 h, and assuming 50% C in litter tissue.

Leaves were air-dried and weighed into 4-g quantities and placed into 6.4-mm mesh litterbags. Eight replicate bags ($n = 8$) were created for each of four treatments at each of five harvest dates for a total of $N = 160$ litterbags. Litterbags were then randomly assigned both a harvest date and a block within the stream. Bags were anchored in the stream along 2-m lengths of steel rebar and wedged into place in active depositional areas near shore. Litterbags were har-

TABLE 1. Initial leaf litter chemistry characteristics and decomposition rate constants (k) for four cottonwood (*Populus*) cross type mixtures (mean \pm SE).

Cottonwood cross type	Condensed tannin (%)	N (%)	P (%)	C:N ratio	k (d ⁻¹)
<i>P. fremontii</i>	0.058 ^a \pm 0.02	0.433 ^a \pm 0.01	0.043 ^a \pm 0.01	99.11 ^b \pm 2.8	0.0162 ^b \pm 0.001
F ₁ hybrid	1.009 ^{ab} \pm 0.16	0.480 ^a \pm 0.01	0.049 ^a \pm 0.01	92.25 ^{ab} \pm 3.1	0.0153 ^b \pm 0.001
Backcross	5.911 ^{ab} \pm 0.50	0.631 ^b \pm 0.02	0.075 ^a \pm 0.01	72.05 ^a \pm 1.9	0.0127 ^a \pm 0.001
<i>P. angustifolia</i>	9.261 ^b \pm 1.80	0.453 ^a \pm 0.01	0.074 ^a \pm 0.01	103.80 ^b \pm 2.2	0.0117 ^a \pm 0.001

Note: Different lowercase letters denote significant differences within a column (Tukey's hsd or Hommel's correction) at an alpha level of 0.05.

vested from the stream after 7, 14, 28, 56, and 83 days. Harvested litterbags were placed into individual polyethylene zipper bags and transported on ice to the laboratory.

Litterbags were processed within 12 h of harvesting. Sediment and invertebrates were sieved through 250- μ m nets for preservation in 70% ethanol. Remaining leaf material was oven-dried at 70°C for 72 h, then ground and combusted at 500°C for 1 h to determine ash-free dry mass (AFDM).

Aquatic invertebrates

Preserved invertebrate samples were sieved through a 1-mm sieve to separate micro- from macroinvertebrates. All invertebrate identifications were made using a dissecting microscope, and aquatic insects (except some members of Diptera) were identified to genus using Merritt and Cummins (1996) and Wiggins (1996), while other invertebrates were identified to the lowest taxonomic level possible using Thorpe and Covich (2001). Reference specimens are maintained in the Marks Aquatic Ecology Laboratory at Northern Arizona University. We identified 28 genera from a total of 24 families and 11 orders.

Statistical analyses

Results are reported as mean \pm SE. Initial leaf chemistry measures for each treatment were analyzed using one-way analysis of variance (ANOVA) and post-hoc comparisons (Tukey's honestly significant difference, hsd). Correlations between continuous variables were analyzed with Pearson's r . Statistical analyses were performed in JMP-IN 4.0.4 (SAS 1989–2001) with an alpha of 0.05.

Analysis of leaf litter decay rates (k) required a natural log-transformation of AFDM remaining for two reasons: (1) to meet normality and equal variance assumptions, and (2) to determine the exponential decay rate (k) of ln AFDM remaining by harvest day (Olson 1963, Petersen and Cummins 1974, Benfield 1996). Decay constants (k) were compared using an equality of slopes test in SAS 8.01 (SAS 1999–2000), and pairwise rate comparisons were corrected using a Hommel's corrected alpha level (Swan and Palmer 2004).

Aquatic invertebrate communities were analyzed using species abundance, species richness, species evenness, Shannon's diversity index (H'), and Simpson's diversity index (D') for each litterbag at harvest dates 7, 28, and 83 d. Values were compared using two-way ANOVA and post-hoc comparisons (Tukey's hsd). To analyze invertebrate community-wide responses to leaf litter treatments, we used a relativized (to species maximum) nonmetric multidimensional scaling (NMDS) ordination method with a Bray-Curtis distance measure in PC-ORD 4.02 (McCune and Mefford 1999), and differences between groups were analyzed using a blocked (by stream location) multi-response permutation procedure (MRBP) in the same program. Seventy-two communities and a total of 52 taxa were compared (see Supplement).

RESULTS

Initial leaf chemistry differed among the four cottonwood cross types. *P. angustifolia* showed significantly higher concentrations of condensed tannins than *P. fremontii* (Table 1). All litter types were indistinguishable in terms of percentage phosphorus in litter, and backcross hybrids showed the highest percentage nitrogen in litter (Table 1). Initial organic carbon content of the four leaf litter treatments differed significantly ($F = 15.78$, $df = 3, 20$, $P < 0.0001$) showing a gradient from low initial organic carbon in *P. fremontii* litter to high initial organic carbon in *P. angustifolia* litter, with intermediate values for both hybrid types. Despite high organic carbon levels for backcross litter, high levels of nitrogen in this litter corresponded to the lowest C:N ratio for this cross type (Table 1).

We found significant differences in decomposition rate constants (k) among cross types (Table 1; see Plate 1). Decomposition rate constants (k) for the two parental species differed significantly and ranged from 0.0117 d⁻¹ for *P. angustifolia* to 0.0162 d⁻¹ for *P. fremontii*. Hybrid rate constants were intermediate to the parental rates, but also significantly different from one another, ranging from 0.0126 d⁻¹ for backcross hybrids to 0.0153 d⁻¹ for F₁ hybrids. Decomposition rate was negatively correlated with percentage condensed tannin

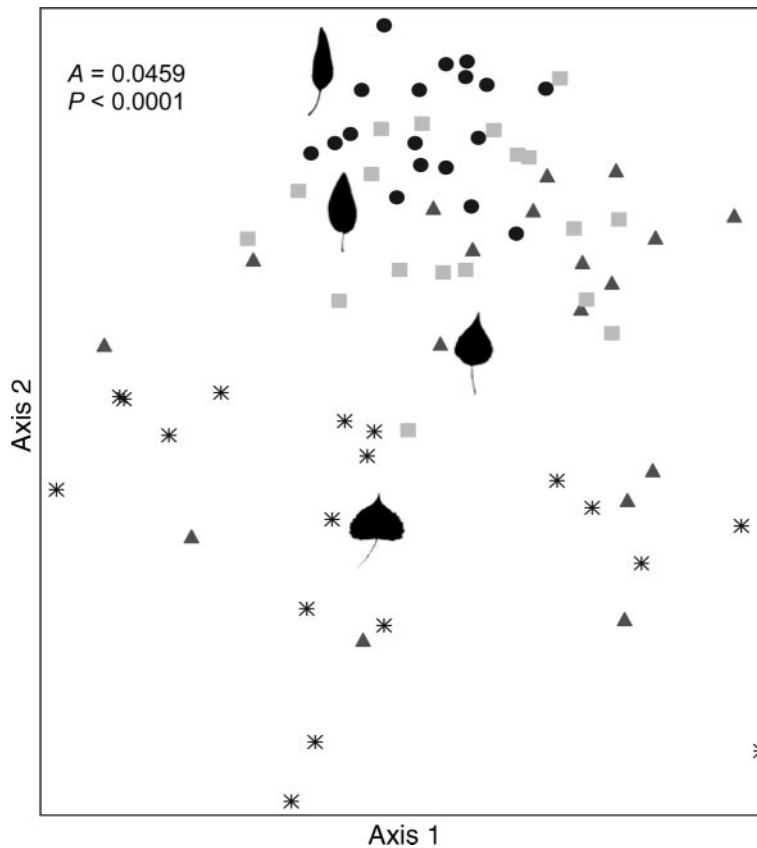


FIG. 1. Nonmetric multidimensional scaling (NMDS) ordination of aquatic invertebrate communities in n -dimensional space. Symbols represent invertebrate communities colonizing *Populus fremontii* (asterisks), *P. angustifolia* (solid circles), F_1 hybrids (gray triangles), and backcross hybrids (gray squares) in leaf litterbags. Whole-model MRBP $A = 0.0459$, $P < 0.0001$. Contrasts reveal that *P. fremontii* litter supports a different aquatic community than litter from hybrids or *P. angustifolia*.

(Pearson's $r = -0.9872$, $P = 0.0128$), and not significantly correlated with any other measured leaf litter chemical parameter.

Total invertebrate abundance, species richness, and species evenness all showed a significant harvest date effect (respectively, $F = 117.3$, $df = 1$, 90 , $P < 0.0001$; $F = 216.91$, $df = 1$, 90 , $P < 0.0001$; $F = 21.71$, $df = 1$, 90 , $P < 0.0001$), but none of these measures showed a significant cross type effect or a significant cross type \times harvest date interaction. Shannon's diversity index (H') and Simpson's D' showed no significant effects of cross type, harvest date, or interaction between them.

Although species richness, evenness, and diversity did not differ among cross types, the composition of the community differed significantly. Using a blocked multi-response permutation procedure (MRBP), we found that aquatic invertebrate communities discriminated among cottonwood parental and hybrid types (Fig. 1). Post-hoc tests revealed that invertebrate com-

munities associated with *P. fremontii* could be distinguished from all other cross types ($A = 0.0459$, $P < 0.0001$). Taxon-specific differences among cross types included twofold greater abundances of *Baetis* sp., *Argia* sp., *Hydropsyche* sp., *Hydroptila* sp., *Physella* sp., *Ferrissia* sp., and Decapoda on *P. fremontii* litter, and twofold fewer *Polycentropus* sp. on *P. fremontii* litter.

DISCUSSION

Litter quality differences and decomposition

Condensed tannin concentration significantly retards decomposition and explains 97% of the variability in decomposition rates for the four cross types. Decomposition rates for *P. fremontii* and F_1 hybrids do not differ although these leaf types differ genetically by 50% of the species-specific genetic markers reported in a previous study (Martinsen et al. 2001). In contrast, the F_1 hybrid and *P. angustifolia*, which also differ genetically by 50% of species-specific markers, de-

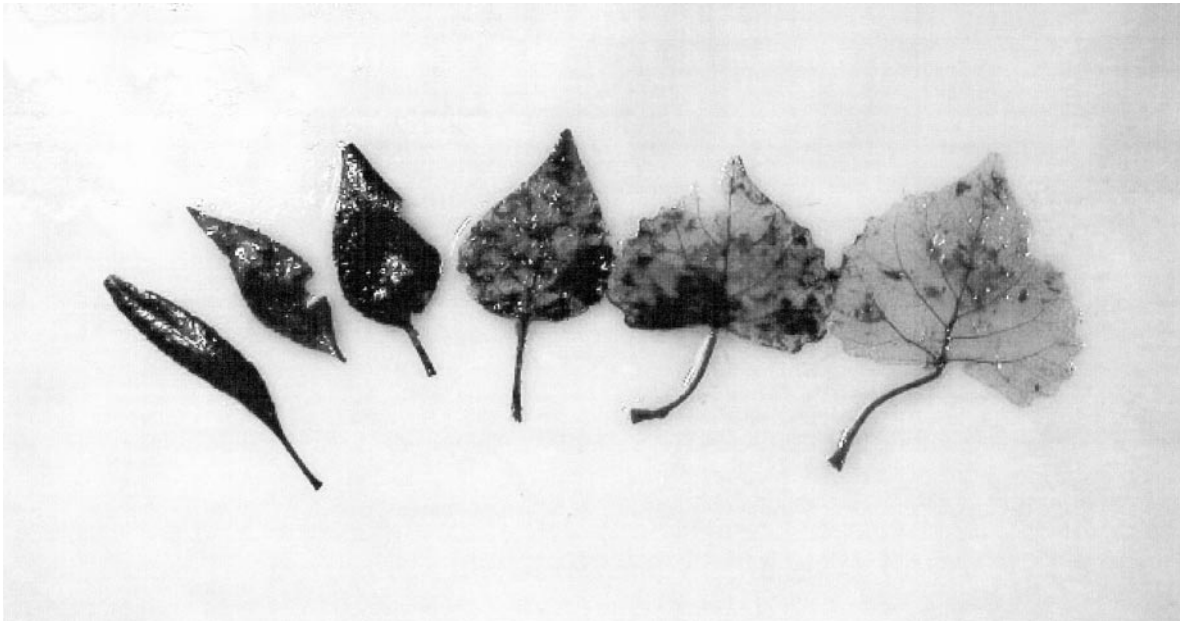


PLATE 1. Cottonwood leaves after a stream incubation of 28 days. Leaf cross types are (from left to right), *Populus angustifolia*, backcross hybrids (2), F_1 hybrid, and *Populus fremontii* (2). The degree of skeletonization increases with increasing proportion *P. fremontii* markers. Photo credit: C. LeRoy.

compose at significantly different rates. It is possible that *P. fremontii* genes are dominant and their phenotypes are more important for aquatic decomposition than *P. angustifolia* genes.

Hybrid introgression appears to result in backcross hybrids with an intermediate or additive rate of decay between the F_1 hybrid and the *P. angustifolia* parent. Our findings are in contrast to previous work that showed slowest rates of aquatic decomposition for backcross hybrids (Driebe and Whitham 2000). This discrepancy could be caused by inter-annual variability, differences between the two study streams used, methodological differences in litterbag mesh size (3 mm compared to 6.4 mm), or differences among genotypes used in backcross and *P. angustifolia* mixtures between the two studies. Also, litterbags, themselves, have been shown to affect decomposition rates by restricting dissolved oxygen and fungal growth (Cummins et al. 1980), but because we standardized methods across treatments, our findings reflect real differences among cross types. However, we realize that litterbags could exaggerate the effects of litter quality on stream invertebrates by excluding other litter types or decrease the effects of litter quality on stream invertebrates by excluding some of the larger shredding invertebrate taxa.

Genetic structuring of the detritivore community

Although standard measures of community structure (e.g., species richness, abundance, and evenness) were

not sensitive to cross type, overall community composition did respond to cross type. *Populus fremontii* hosted a unique assemblage of invertebrates compared to the other cross types (Fig. 1) even though *P. fremontii* and F_1 hybrid leaf litter decomposed at indistinguishable rates (Table 1). Aquatic communities are therefore likely responding to genetic-based differences in polyphenolic concentrations or polyunsaturated lipids, which could be more variable in litter than measured rates of instream decomposition (Hanson et al. 1985, Driebe and Whitham 2000, Schweitzer et al. 2004).

While the overall invertebrate community colonizing *P. fremontii* litter was significantly different than the communities colonizing the other three cross types, the individual invertebrate taxa that were driving this difference were not expected. Members of the shredder feeding guild were abundant, but other than decapods, shredders did not appear to discriminate among litter types. However, members of other feeding guilds did discriminate, specifically scrapers, collectors, grazers, and predators.

Ecological and evolutionary implications

This research has broad implications for riparian restoration and stream trophic dynamics because it shows that aquatic invertebrates can distinguish among trees at a much finer genetic scale than previously thought, and potentially through indirect trophic pathways. *Po-*

pulus species and their naturally occurring hybrids (and other hybridizing species such as *Salix* sp.) are often pooled in analyses of instream decomposition and aquatic detritivore community responses (e.g., Casas et al. 1994, Essafi et al. 1994) even though the phytochemistry of these trees can vary as much as different plant families (e.g., Pinaceae and Aceraceae, data not shown).

Our findings of the effects of plant genetics on aquatic arthropod community structure are similar to findings in the same system for the terrestrial arthropod community, but not as strong. Wimp et al. (2005) found that arthropod species richness and abundance did not differ significantly among cross types, but found significantly different communities on the different cross types (*P. fremontii* ≠ F₁ hybrid ≠ backcross hybrid = *P. angustifolia*). We predicted that the cascading effects of terrestrial plant genes would become more diffuse with increasing distance from the source, i.e., a genetic diffusion hypothesis. Specifically, we expected that terrestrial arthropods on living trees would be more affected by the genetic composition of their hosts than aquatic organisms living on the leaf litter in a nearby stream, and this appears to be the case.

This study, in combination with other studies in this system, shows that the genetic variation within a naturally hybridizing complex can affect diverse taxa occupying multiple trophic levels, such as fungal, arthropod, avian, and mammalian herbivores, decomposers, predators, and parasites (Martinsen and Whitham 1994, Dickson and Whitham 1996, Whitham et al. 2003, Bailey et al. 2004, Wimp et al. 2005).

Riparian restoration projects often involve the revegetation of slopes adjacent to rivers with single clones of *Populus* (Winfield and Hughes 2002). The continued use of these clones to restore riparian forests could lead to the loss of aquatic species diversity and trophic destabilization through the loss of riparian genetic diversity (see also Wimp et al. 2004). Similarly, the introduction of new genes to riparian ecosystems through transgenic cottonwood plantings could have unpredictable effects on stream processing capacities and linked aquatic communities. Poplars cloned for pulp production are being genetically manipulated to resist herbicides (Meilan et al. 2002) as well as terrestrial herbivores (Wang et al. 1996). The ecosystem consequences of these practices are unknown, making it imperative to understand the ecological consequences of genetic variation on ecosystem function.

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SUPPLEMENT

Macroinvertebrate community matrix data (*Ecological Archives* E087-012-S1).