Freshwater Biology

Freshwater Biology (2016) doi:10.1111/fwb.12872

It is about time: genetic variation in the timing of leaf-litter inputs influences aquatic ecosystems

MARIANO A. RODRIGUEZ-CABAL*,†, M. NOELIA BARRIOS-GARCIA†,‡, SETH M. RUDMAN†, ATHENA D. MCKOWN§, TAKUYA SATO¶ AND GREGORY M. CRUTSINGER†

*Grupo de Ecología de Invasiones, INIBIOMA - CONICET, Universidad Nacional del Comahue, Bariloche, Rio Negro, Argentina

†Department of Zoology, University of British Columbia, Vancouver, BC, Canada

‡CONICET, CENAC – APN, Bariloche, Rio Negro, Argentina

§Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada

¶Department of Biology, Graduate School of Science, Kobe University, Kobe, Japan

SUMMARY

1. Phenology, or the timing of life cycle events, is a key trait of organisms that has significance for how communities are assembled and ecosystems function. Although variation in phenology in plants has received increased attention over the past decade as a result of changing climate, we are only beginning to understand the role of genetic variation in these phenological traits on ecological interactions and ecosystem-level processes.

2. The influence of tree species on riparian environments presents an interesting system for understanding the effects of phenology in terrestrial species on aquatic ecosystems. Here, we used a dominant riparian tree (*Populus trichocarpa*: Salicaceae) and tested intraspecific genetic variation in the phenological timing of leaf drop, which influenced leaf-litter inputs into our experimental aquatic ecosystems.

3. Our empirical results found that genotypic differences in *P. trichocarpa* explained much of the variation both in leaf-litter decomposition and aquatic invertebrate species richness within our experimental ponds. Moreover, our results showed that variation in the timing of leaf-litter inputs outweighed the effects of variation in leaf-litter quality among *P. trichocarpa* genotypes on aquatic invertebrate species richness.

4. Taken together, our results suggest that genetic variation in the timing of litter inputs from dominant plant species is likely to be a strong underlying mechanism driving litter decomposition and invertebrate communities in aquatic ecosystems. This emphasises that studies disregarding phenology may significantly underestimate an important and variable component in communities and ecosystems.

Keywords: aquatic-terrestrial linkages, community genetics, insect diversity, phenology, *Populus trichocarpa*

Introduction

Intraspecific genetic variation has long been recognised for its importance as the raw material for natural selection and evolution within species (Fisher, 1930). There has been growing appreciation in ecology for the role of intraspecific genetic variation, particularly within dominant and foundation plants species, in shaping associated communities and driving ecosystem processes (Hughes & Stachowicz, 2004; Whitham *et al.*, 2006; Johnson & Stinchcombe, 2007; Bailey *et al.*, 2009; Crutsinger *et al.*, 2014a). For example, genetic relatedness predicts primary productivity among eelgrass (*Zostera marina*) clones in intertidal ecosystems (Stachowicz *et al.*, 2013), while variation among aspen (*Populus tremuloides*) clones can influence the composition of soil microbes (Madritch & Lindroth, 2011). Although there has been significant progress in identifying the effects of genetic variation on

Correspondence: Mariano A. Rodriguez-Cabal, Grupo de Ecología de Invasiones, INIBIOMA - CONICET, Universidad Nacional del Comahue, Av. de los Pioneros 2350, Bariloche, Rio Negro CP. 8400, Argentina. E-mail: rodriguezcabal@gmail.com

higher levels of ecological organisation, most studies have not identified the underlying traits driving these ecological responses (Hughes *et al.*, 2008). Of the studies that have used a trait-based approach, most have focused primarily on traits related to plant productivity, nutrient content and secondary defences (Whitham *et al.*, 2012), yet there are likely genetic differences in other traits that are potentially relevant to communities and ecosystems.

Phenology, or the timing of life cycle and seasonal events, is a fundamental aspect of organisms that has profound consequences for ecological interactions and processes (such as pollination, pupation, mating, seasonal pulses of leaf litter) (Revilla, Encinas-Viso & Loreau, 2014). Within species, there can be considerable genetic variation in phenology (Moore & Schindler, 2010). For example, the timing of emergence of mayfly larvae from aquatic environments can vary considerably among populations (Brittain, 1982). Within plant species, spring flowering time can vary from days to weeks within and among plant populations (Primack, 1980), while canopies of different aspen (*P. tremuloides*) genets growing adjacent to one another can be at very different phenological stages, ranging from green to bright yellow or orange, during autumnal senescence (Lindroth & Hwang, 1996). In addition, leaf flushing and senescence are related to plant primary productivity, define the length of biogeochemical cycling, and the dynamics of carbon sequestration (Morisette *et al.*, 2009; Polgar & Primack, 2011). Although it is well established that there is a considerable amount of phenological variation within species, we are only beginning to understand the role of genetic variation in phenology in structuring the diversity and composition of associated communities or driving ecosystem-level processes (Burkle, Marlin & Knight, 2013; Revilla *et al.*, 2014). Predicting the timing of recurrent life cycle events in current scenarios of climate change is essential to evaluate, manage and mitigate its consequences (Harper & Peckarsky, 2006; McNamara *et al.*, 2011; Johansson & Jonzen, 2012; Reed, Gienapp & Visser, 2015).

Most studies on the ecological consequences of genetic variation have been performed using terrestrial plants and terrestrial communities, while only a few studies have looked at the cross-ecosystem effects of genetic variation using litterbags placed in streams (LeRoy *et al.*, 2006, 2007; Jackrel & Wootton, 2015). These studies have concentrated exclusively on traits related to litter quality, such as secondary chemistry, ignoring genetic variation in other traits that might influence aquatic–terrestrial interactions. For example, LeRoy *et al.* (2006, 2007) examined leaf litter from two *Populus* parental species (*P. fremontii* and *P. angustifolia*) and their hybrids and found differences both among parental species and hybrids driven primarily by variation in condensed tannins. In this study, we examined the effects of intraspecific genetic variation in phenology of leaf litter fall in black cottonwood (*Populus trichocarpa*), a dominant riparian tree species in western North America, on experimental aquatic ecosystems. Leaf litter is a fundamental carbon resource in riparian zones, acting as a key linkage between terrestrial and aquatic ecosystems (Marcarelli *et al.*, 2011). Seasonal leaf-litter inputs into adjacent streams, lakes and ponds can have strong effects on food-web dynamics and nutrient cycling (Wallace *et al.*, 1997; Gessner *et al.*, 2010), and the timing of the resource pulses can be critical to understanding how aquatic ecosystems function (Takimoto, Iwata & Murakami, 2002; Holt, 2008; Yang *et al.*, 2008; Leroux & Loreau, 2012).

Here, we present empirical results from a large field experiment consisting of a common garden containing different *P. trichocarpa* genotypes growing adjacent to created aquatic pond mesocosms. This research is particularly relevant given rising concern over shifts in the phenology of many species and the timing of the interactions under a changing climate (Yang & Rudolf, 2010). Previous goals of this experiment addressed how genetic variation influences aquatic-terrestrial linkages (Crutsinger *et al.*, 2014a; Rudman *et al.*, 2015), whereas the results presented here are unique in that they focus on the role of timing of leaf-litter inputs. We aimed to explicitly investigate the impact of genetic variation in leaf senescence in *P. trichocarpa*, and the subsequent leaf-litter inputs to aquatic systems with the hypothesis that variation in the timing of leaf-litter inputs to the aquatic system would play a larger role in dictating decomposition than variation in leaf-litter quality among genotypes. We predicted that qualitative differences in leaf-litter inputs among tree genotypes would be the underlying mechanism driving invertebrate community responses when litter inputs occur at the same time. However, it was unclear whether incorporating the genetic variation in tree phenology (affecting the timing of leaf-litter inputs) would alter the magnitude or direction of ecological responses. For example, shifts in the aquatic community associated with changes during autumn could influence whether the effects of genetic variation in litter are maintained or not. Despite the complexity of predicted outcomes, our study provides a novel perspective of the consequences of genetic variation in tree phenology for aquatic-terrestrial linkages.

Methods

Study system

Our experimental setup was designed to mimic the small pools and ponds in the riparian ecosystems of British Columbia (BC), Canada, where a small pond might receive the majority of its leaf-litter inputs from an adjacent adult tree. *Populus trichocarpa* is often the dominant tree species in riparian ecosystems in western North America, ranging from California to southern Alaska (Farrar, 1995). With a fully sequenced genome and extensive genetic resources, it is also a model organism for understanding the genetic basis of phenotypic variability in woody species (Cronk, 2005). Levels of genetic differentiation among populations of *P. trichocarpa* are low throughout the species range due to high gene flow through wind pollination and seed dispersal (Wegrzyn *et al.*, 2010; Slavov *et al.*, 2012; Geraldes *et al.*, 2014); yet, populations maintain high levels of heritable phenotypic differentiation from a strong genetic component in many traits reflecting adaptation to local environmental conditions (Weber, Stettler & Heilman, 1985; Xie, Carlson & Ying, 2012; McKown *et al.*, 2014a).

In 2008, 461 *P. trichocarpa* genotypes originating from 136 provenance localities throughout the species range were planted in a common garden at Totem Field, University of British Columbia (UBC), Vancouver, BC (McKown *et al.*, 2013). This common garden was genotyped (Geraldes *et al.*, 2013) and extensively phenotyped, including measures of a wide range of biomass, ecophysiology and phenological traits that were assessed across multiple years (McKown *et al.*, 2013, 2014a,b,c). These studies determined population structure and trait heritability within the collection, related variation in these traits to gradients in the geography and environment of tree origin (i.e. geoclimate variables), and assessed the genetic architecture underlying these phenotypes. Within the *P. trichocarpa* collection, McKown *et al.* (2014a,c) found a large amount of heritable variation in traits relating to litter quality, such as litter nutrient and tannin content, and variation in these traits was shown to influence ecological processes in an experimental aquatic ecosystem (Crutsinger *et al.*, 2014a). Variable phenological canopy traits, such as timing of bud break, leaf flush, bud set, leaf yellowing (senescence) and leaf drop (creating leaf litter), were also highly heritable in *P. trichocarpa* (McKown *et al.*, 2014a). The late summer and autumn phenology traits (i.e. bud set, leaf senescence and leaf drop) strongly reflect tree origin in *P. trichocarpa* and ultimately control the timing of litter inputs. The primary cues for each of these events are a combination of temperature and photoperiod, which can act together or independently with photoperiod being the predominant cue (McKown *et al.*, 2014a). Genome-wide association studies have shown that the genetic basis underlying these phenological traits is complex, involving multiple genes with each gene explaining only a small proportion of the heritable phenotypic variation (McKown *et al.*, 2014b). The ecological consequences of genetic variation in phenology traits that drive litter inputs have not been explored in *P. trichocarpa*, and therefore, our study focuses on genetic variation in phenology as the underlying mechanism modifying leaf-litter inputs under a common environment and not on individual genes *per se*. Although relationships among phenological traits and geoclimatic variables have been previously discussed within a species adaptation context (McKown *et al.*, 2014a), this study provides a unique perspective for how these patterns might influence litter-associated communities, relate to aquatic subsidies and affect decomposition.

Experimental design

In March 2012, five *P. trichocarpa* genotypes (hereafter referred to as G1–G5) were selected from a pool of 269 candidates to represent the full range of variability in phenology among southern BC localities (latitudinal range: 49–52°N). All five genotypes were from different provenances located within southern BC and were considered equally genetically distant (Crutsinger *et al.*, 2014a; Geraldes *et al.*, 2014). Dormant whips were taken from trees grown in Totem Field, and cuttings cultivated within a greenhouse setting and transplanted into individual 94.6-L plastic nursery containers (details in Crutsinger *et al.*, 2014a). Trees were fertilised at the time of planting and watered as needed throughout the duration of the experiment. In June 2012, each of the five *P. trichocarpa* genotypes were grown in monoculture around experimental aquatic pond mesocosms (complete methodological details on the common garden and aquatic mesocosms setup are in Crutsinger *et al.*, 2014a). Briefly, we planted three replicated ramets around each mesocosm and 12 replicate mesocosms per genotype (180 trees and 60 mesocosms total; Fig. 1). Mesocosms were constructed from 1136-L cattle tanks (2 m in diameter, 1 m in depth; Rubbermaid Commercial Products, LLC, Winchester), spaced 3 m apart and randomly assigned to a location in a 30 × 100 m grid on the UBC research farm. Each tank was filled with well water, and 11.33 kg of sterilised play sand was added to create a sediment layer. We inoculated each mesocosm with

Fig. 1 Photo of randomised experimental array showing clonal genotypes of black cottonwood, *Populus trichocarpa*, surrounding aquatic mesocosms (photo credit: G. Crutsinger).

phytoplankton and zooplankton taken from nearby experimental ponds. We also introduced a microbial community and propagules of benthic and pelagic organisms by adding sieved benthic mud from a nearby shallow lake (Browning Lake, Squamish). Mesocosms were then left uncovered throughout the summer to allow for the natural colonisation of other aquatic invertebrates. We added a small amount of nutrients (1.23 g $NaNO_3$ and 0.09 g NaH_2PO_4) to each tank to boost initial primary production, and supplemental well water was added throughout the summer to compensate for evaporation.

Leaf-litter manipulation

In the autumn period prior to the start of leaf drop (late September 2012), we bagged all *P. trichocarpa* individuals using netting staked with a 3-m bamboo pole. Consistent with their variable autumn phenology observed in prior studies (McKown *et al.*, 2013, 2014a), *P. trichocarpa* genotypes varied in timings of canopy senescence and peak leaf drop (creating litter for our experiment) in our study. All genotypes had dropped enough leaf litter by early October 2012 to initiate the decomposition experiment presented here. Litter was collected, air-dried for 48 h, weighed, and 75% of the leaf mass was deposited directly into the corresponding mesocosm as part of a larger whole-ecosystem experiment (Crutsinger *et al.*, 2014a). The remainder of the litter was used to estimate nutrient content and provided material for this study. Using the leaf litter collected in early October 2012, we created two decomposition litterbags for each mesocosm (60 mesocosms × 2 litterbags = 120 litterbags total). Decomposition bags (15 × 15 cm) were constructed of window screen (2-mm mesh size). Bags were sealed on three edges using an impulse heat sealer (Grizzly Industrial Inc., Bellingham), filled with 2 g of dried *P. trichocarpa* litter (amassed from all three individuals for each mesocosm) and sealed on the fourth edge. We established two separate litter input manipulations. First, we placed a single litterbag into all mesocosms on the same date. We refer to this as the 'litter quality' experiment, as the timing of litter input was equivalent allowing only variation in litter quality. We placed these first litterbags in our mesocosms on 2 October as the earliest *P. trichocarpa* genotype (G1) was already reaching peak litter fall (*c.* 75% of the canopy had dropped). The second experiment placed the other litterbag into the corresponding mesocosm at different times and during the peak litter fall for a given tree genotype (estimated through visual surveys of proportion of leaves remaining on trees taken on a weekly basis). We refer to this as the 'litter timing' experiment, as both genetic variation in litter quality and timing were present in this manipulation. Placement of the second litterbag occurred on 2 October for G1 (same date as the 'litter quality experiment'), *c.* 2 weeks later (18 October) for G2 and G3, and *c.* 4 weeks later (31 October) for G4 and G5.

All litterbags were fully submerged within the mesocosms during which time they rested flat on the bottom of each tank. After 30 days, litterbags were collected and litter assessed for benthic invertebrates (see below). Litter was then oven dried at 60 °C for 72 h and weighed. Subsamples of dried litter were ground to a fine powder using a ball mill (Spex 8000D; SPEX SamplePrep, Metuchen) and ashed in a muffle furnace at 550 °C for 6 h. All litter mass loss data are shown as ash-free dry mass. To measure litter carbon (C) and nitrogen (N) content, 5–6 mg of ground material was weighed on a microbalance (Metter XP6; Mettler Toledo, Columbus) and analysed for nutrient content using an elemental analyser (Costech ECS 4010; Costech Analytical Technologies Inc., Valencia) at UBC with acetanilide (10.36% N and 71.09% C) as a reference standard. To measure litter phosphorus (P) content, 5–6 mg ground material was ashed at 500 °C for 2 h to remove all organic matter. Ashed material was then digested in a 1 N HCL solution at 105 °C for 2 h. Dissolved P in the digested material was measured using the colorimetric molybdate method (Murphy & Riley, 1962). Absorbance was measured with a 1240 mini-Shimadzu spectrophotometer (Shimadzu, Sidney), and spinach leaves (NIST #1570a) were included as internal standards. Our extraction efficiency was estimated as *c.* 99% (CoV < 5%), and variability between replicated samples was small (CoV < 6%). To

determine soluble condensed tannin concentrations, we used the butanol–HCl method (Porter, Hrstich & Chan, 1986). Complete methodological details on condensed tannin are in Crutsinger *et al.* (2014a,b).

We note that the quality of leaf litter may change naturally as autumn progresses and that our study does not encapsulate this change by using litter collected earlier in the season for all litterbags. However, we consider it is likely that litter quality declines over time, and further exacerbates differences among *P. trichocarpa* genotypes with earlier versus later autumn phenology. Therefore, we posit that our consistent use of early-season litter for all genotypes represents a more comparable, if more conservative, estimate of leaf-litter quality and decomposition differences among *P. trichocarpa* genotypes relating to phenology.

Benthic invertebrates

We assessed the benthic invertebrates from litterbags used in the decomposition study for taxa richness, abundance and total invertebrate biomass. After litterbags were pulled from mesocosms, we placed them in plastic bags and immediately returned to the laboratory. All leaves were then gently rinsed individually with water into a sorting tray. Benthic invertebrates were picked live and placed into 95% ethanol. Individuals were counted and identified to the lowest feasible taxonomic unit (species or morphospecies). Benthic invertebrates were then oven dried at 60 °C for 72 h to obtain total invertebrate biomass attributable to each litterbag.

Statistical analyses

To examine how differences in the timing of litter inputs influenced litter decomposition and benthic invertebrates associated to litterbags (taxa richness, abundance and biomass), we used separate full-factorial analysis of variance (ANOVA) examining genotype, litter input timing (litter quality versus litter timing), and genotype × litter input timing interaction. We performed Tukey HSD *post hoc* pairwise comparisons to identify significant differences between groups ($P < 0.05$). We used linear regressions to examine how litter traits (condensed tannins, C:N and P) related to litter decomposition. Finally, to determine how arthropod community composition varied with plant genotypes and sampling times, we used *t*-test and separate two-way ANOVAs to examine variation in mayfly (Ephemeroptera) and chironomid (Diptera) abundance across treatments, as these were the dominant invertebrates in our samples, and PERMANOVA using the Bray-Curtis dissimilarity matrix on log-transformed abundance data (Primer-E version 1.0.5, Primer-E, Auckland). Data were log-transformed as needed to improve normality and reduce heteroscedasticity. For clarity and ease in discussing the results, we show the untransformed values in all figures.

Results

We found that variation in leaf-litter inputs among different *P. trichocarpa* genotypes strongly influenced litter decomposition (Table 1). When litterbags were established simultaneously ('litter quality' experiment), plant genotype explained half of the variation in decomposition ($R^2 = 0.53$, $F_{4,52} = 14.46$, $P < 0.0001$). *Populus trichocarpa* genotypes varied by 23% in litter mass loss and

Table 1 Results from two-way ANOVAs examining the effects of genetic variation in *Populus trichocarpa* leaf-litter input timing on litter decomposition and benthic invertebrates associated with litterbags (taxa richness, abundance and total biomass). The litter input timing variable represents either simultaneous input (2 October) or input dependent on the peak litter fall for each genotype (2, 18 or 31 October). Bold indicates statistically significant differences ($P < 0.05$).

Source	d.f.	*F*	*P*
Litter mass loss (%)			
Model	9,104	52.94	**<0.0001**
Genotype	4	35.44	**<0.0001**
Litter input timing	1	214.37	**<0.0001**
Genotype × litter input timing	4	27.84	**<0.0001**
Total richness			
Model	9,100	4.30	**<0.0001**
Genotype	4	1.88	0.119
Litter input timing	1	26.12	**<0.0001**
Genotype × litter input timing	4	0.67	0.612
Total abundance			
Model	9,96	1.88	0.064
Genotype	4	0.46	0.763
Litter input timing	1	0.92	0.340
Genotype × litter input timing	4	3.31	**0.014**
Total biomass (g)			
Model	9,109	2.53	**0.011**
Genotype	4	2.73	**0.033**
Litter input timing	1	3.12	0.080
Genotype × litter input timing	4	2.17	0.077
Mayfly abundance			
Model	9,86	1.87	0.067
Genotype	4	0.38	0.821
Litter input timing	1	6.21	**0.016**
Genotype × litter input timing	4	2.95	**0.025**
Chironomid abundance			
Model	9,86	0.76	0.655
Genotype	4	0.51	0.730
Litter input timing	1	1.55	0.220
Genotype × litter input timing	4	0.81	0.525

overall mass loss averaged 39% across all genotypes during this time period, with a rank order of mass loss (least to most) of G2 < G1 < G5 < G4 < G3 (Fig. 2). We found substantial variation in litter quality. Mean litter P content varied by 30% ($F_{4,55}$ = 11.70, P < 0.0001), leaf C:N varied by 200% ($F_{4,51}$ = 35.58, P < 0.0001) and condensed tannins by 900% ($F_{4,35}$ = 11.70, P < 0.0001). When we examined the traits that best accounted for *P. trichocarpa* litter decomposition when litterbags were introduced simultaneously, we found that the C:N ratio accounted for 16% of the variation in litter decomposition, while condensed tannin content did not explain litter decomposition (Fig. 3).

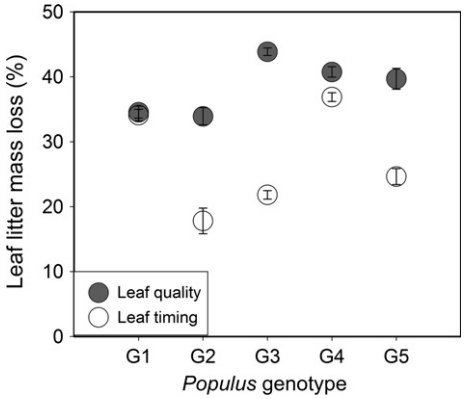

Fig. 2 Percent litter mass loss from 30-day decomposition experiments ('litter quality' and 'litter timing') by *Populus trichocarpa* genotype. Grey represents litterbags added to mesocosms simultaneously (testing litter quality effects), white represents litterbags added to mesocosms during peak litter fall (loss of 75% of the tree canopy) and bars indicate SE within genotypes.

When litterbags were established during peak litter fall for a given plant genotype ('litter timing' experiment), plant genotype explained substantially more of the variation in decomposition (R^2 = 0.74, $F_{4,52}$ = 36.64, P < 0.0001). *Populus trichocarpa* genotypes varied by over 200% in litter mass loss in the litter timing experiment (Fig. 2). On average, litter established later decomposed more slowly, averaging 27% mass loss overall. Moreover, there was a different rank order of mass loss (least to most) when litterbags were established during peak litter fall G2 < G3 < G5 < G1 < G4 compared to litterbags established simultaneously (Fig. 2). Peak timing of litter fall (>75% canopy loss) varied by *c.* 30 days among genotypes (Fig. 3), and when we included the 'start date' when the litterbags were added to the mesocosms as a factor, we found that this date (Fig. 3a) was the most important variable explaining 64% of the total variation in litter mass loss for litterbags during peak litter fall. In general, the later litterbags were established in mescosms, the less litter mass was lost, despite being submerged for the same amount of time.

We found that variation in leaf-litter inputs relating to leaf quality and timing significantly altered the richness, abundance and biomass of benthic invertebrates associated with leaf litter. In total, we found 17 different morphospecies (mean ± SE, 2.19 ± 0.09 per genotype) and 1632 individuals (mean ± SE, 14.84 ± 1.79 per genotype). The litterbags added at the time of peak litter fall

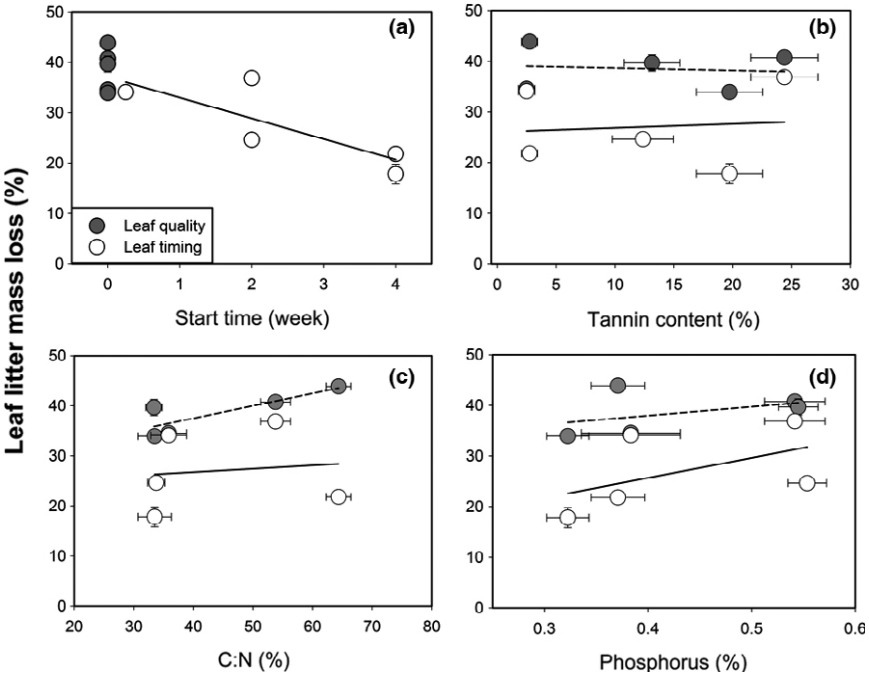

Fig. 3 The plant traits that best accounted for *Populus trichocarpa* litter decomposition as described by percent litter mass loss. (a) Initial time (week) when litter was added during peak litter fall (R^2 = 0.64, P < 0.001), (b) condensed tannin content (%) (litter quality: R^2 = 0.01, P = 0.880; litter timing: R^2 = 0.001, P = 0.952), (c) carbon: nitrogen content (C:N) (%) (litter quality: R^2 = 0.67, P = 0.091; litter timing: R^2 = 0.016, P = 0.840) and (d) phosphorus (P) (%) (litter quality: R^2 = 0.21, P = 0.442; litter timing: R^2 = 0.29, P = 0.341). Grey represents litterbags added to mesocosms simultaneously (testing litter quality effects), white represents litterbags added to mesocosms during peak litter fall (loss of 75% of the tree canopy) and bars indicate SE within genotypes.

had *c.* 28% lower taxa richness (across all genotypes) compared with litterbags which were added simultaneously. The timing of leaf-litter inputs also had a significant interaction with tree genotype that influenced the total abundance of benthic invertebrates. Specifically, some *P. trichocarpa* genotypes showed greater invertebrate abundance when litterbags were added during peak litter fall (e.g. G1 had 77% greater invertebrate abundance), while others showed a reduction (e.g. G4 had 59% lower abundance). Differences in litter quality among *P. trichocarpa* genotypes also had an effect on the biomass of benthic invertebrates. For instance, the G5 genotype had 450% more invertebrate biomass than the G1 genotype when litterbags were added to mesocosms at the same time ($F_{4,55} = 3.11$, $P = 0.022$). However, we found that the difference between genotypes disappeared when leaf litter was added during peak litter fall ($F_{4,55} = 0.74$, $P = 0.567$), suggesting that both litter quality and the timing of litter inputs play important and interactive roles in shaping the benthic invertebrate community (Table 1).

We assessed two groups of dominant benthic invertebrates (chironomids and mayflies) that together represented >85% of the total abundance of benthic invertebrates. The abundance of chironomids remained equivalent across experiments, as this group represented 50% of all benthic invertebrates when leaf litterbags were added at the same time, and 43% when litterbags established later ($t_{94} = -1.15$, $P = 0.254$). By contrast, the abundance of mayflies varied depending on timing of litterbag establishment, with an average of *c.* 45% fewer individuals per litterbag earlier in the season (e.g. 2 October establishment, 28% of the total abundance benthic invertebrates) compared to when litterbags established later (e.g. 28 October establishment, 51% of the total abundance benthic invertebrates, $t_{94} = 2.06$, $P = 0.042$). These differences may have primarily reflected seasonality as arthropod community composition varied with the time of leaf-litter inputs ($_{\text{Pseudo}}F = 13.72$, $P = 0.0001$).

Discussion

In this study, we observed that genetic variation in timing of leaf drop among *P. trichocarpa* genotypes had clear, significant effects on the litter decomposition in aquatic ecosystems. For example, decomposition of the five genotypes varied by 26% on average, when litterbags were established simultaneously (testing litter quality), but by 200% when litterbags were placed during the given genotype's peak litter fall (testing litter timing). Incorporating the phenological differences among *P. trichocarpa* genotypes magnified the effects of genetic variation on litter decomposition and altered the rank order of genotypes in litter mass loss over time. These results demonstrate that the genetic differences in the phenological timing of canopy loss (defining peak litter fall driving litter inputs) may be a crucial underlying mechanism driving litter decomposition that has been overlooked in ecosystem studies. Researchers often collect litter over the period of senescence and set out litterbags in the field at the same time. While these methods capture qualitative differences in litter due to variation in nutrient and secondary chemical content (Madritch & Hunter, 2002; Schweitzer *et al.*, 2005; Crutsinger *et al.*, 2006; Madritch, Donaldson & Lindroth, 2006), they may significantly underestimate the overall importance of genetic variation in the phenology of dominant plant species for communities and ecosystems.

Surprisingly, when we examined the relationships between key litter quality traits and litter decomposition (as measured by mass loss) in *P. trichocarpa*, we did not find secondary compounds (condensed tannins) to be significant in explaining variation in litter break down. This was despite that condensed tannin content in the five genotypes used in this study varied several-fold (*c.* 3–24% dry leaf weight). Prior work by Schweitzer *et al.* (2005) found that condensed tannins accounted for a considerable amount of the variation in litter decomposition across a cottonwood hybrid zone. The differences observed between these studies could be largely due to ecological context, as leaf-litter decomposition can be influenced by both the microbial and invertebrate species present (Hieber & Gessner, 2002). When we incorporated the influence of phenology in litter inputs, we found that peak litter fall explained a substantial amount of variation in litter mass loss among the five clones. As leaves drop later into the autumn, temperatures may be cooler with fewer invertebrates in the leaf litter, thereby slowing decomposition. In our study, the peak timing of litter fall varied by *c.* 14 days among genotypes G2–G5, and the variation in decomposition among genotypes did show clear patterns in decomposition relating to advancing time (Fig. 3).

Phenological traits affecting litter input timing, such as leaf yellowing and leaf drop, are highly heritable ($H^2 = 0.6$) in *P. trichocarpa* similar to other qualitative litter characteristics, such as condensed tannins ($H^2 = 0.6$), but substantially higher than nitrogen-related traits content ($H^2 = 0.2$) (McKown *et al.*, 2014a,c). Phenological differences among *P. trichocarpa* genotypes are driven primarily by local adaptation to day length, which is unchanging from year to year, but also respond to

seasonal temperature conditions (McKown *et al.*, 2013, 2014a). These abiotic factors vary with latitude and will likely influence an aquatic ecosystem directly (such as affecting rates of decomposition), as well as interacting with genetic effects from dominant plant species phenology. While these G × E interactions (beyond temporal environmental changes, Fig. 3) were out of the scope of this study, our results showing differences in decomposition rates of a given genotype with time suggest that there are clear opportunities for reciprocal transplant experiments that partition the effects of genetic variation in dominant species and community and ecosystem interactions under different environmental conditions. These studies could explore how changes in both temperature and heritable phenotypes would alter community composition and ecosystem function. We would expect that the high heritability of phenological traits would allow for more predictable ecosystem responses to genetic variation in the timing of interactions compared to less heritable traits such as litter nutrient content which might depend on highly localised soil conditions.

In addition to amplifying the differences in decomposition among genotypes, benthic invertebrates also showed a response to genetic variation in leaf-litter inputs. Notably, patterns of benthic invertebrate biomass differed between the litter quality and litter timing experiments. Species abundance and total biomass were significantly affected by plant genotype, and species richness and abundance had significant effects from litter input timing. This suggests that findings from studies that ignore the role of intraspecific variation in phenology may differ from a more realistic scenario where phenology is allowed to vary. Future work to assess the role of intraspecific variation in plant phenology of dominant species in altering trophic interactions, and potentially trophic cascades, will be particularly useful in ecology and its applications. For example, developing a better understanding of the relationship between plant phenology and local biotic interactions could lead to a useful tool for ecosystem management or restoration.

Variation in phenology has received increased attention over the past decade as a result of interactions under a changing climate (Yang & Rudolf, 2010). We suggest that the intraspecific variation in phenology of dominant plants species could be a critical source of intraspecific variation in shaping ecosystem function and ecological communities. Our results show that variation in the timing of autumn phenology (driving litter inputs) within a dominant riparian tree can affect the adjacent aquatic habitat. We would similarly expect that shifts in phenology (such as lengthened summer temperature periods delaying litter inputs) to have important consequences for aquatic–terrestrial interactions. Future work should take into account the phenological timing of leaf inputs as it is a critical underlying mechanism driving litter decomposition. More broadly, understanding the ecological importance of genetic differences in phenology of dominant species in a wide range of ecosystems could lead to a better understanding of how shifting phenologies will alter ecological dynamics.

Acknowledgments

GMC was supported by a NSERC Discovery grant and the Canadian Foundation for Innovation. TS was supported partially by Grant-in-Aid for Young Scientists (A) (grant number: 24687003) and the Hakubi Project, Kyoto University. The phenotyping and genetic association work was supported by the Genome Canada Large-Scale Applied Research Project (Project 168BIO) funds.

References

Bailey J.K., Hendry A.P., Kinnison M.T., Post D.M., Palkovacs E.P., Pelletier F. *et al.* (2009) From genes to ecosystems: an emerging synthesis of eco-evolutionary dynamics. *New Phytologist*, **184**, 746–749.

Brittain J.E. (1982) Biology of mayflies. *Annual Review of Entomology*, **27**, 119–147.

Burkle L.A., Marlin J.C. & Knight T.M. (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science*, **339**, 1611–1615.

Cronk Q.C.B. (2005) Plant eco-devo: the potential of poplar as a model organism. *New Phytologist*, **166**, 39–48.

Crutsinger G.M., Collins M.D., Fordyce J.A., Gompert Z., Nice C.C. & Sanders N.J. (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, **313**, 966–968.

Crutsinger G.M., Rudman S.M., Rodriguez-Cabal M.A., Mckown A.D., Sato T., Macdonald A.M. *et al.* (2014a) Testing a 'genes-to-ecosystems' approach to understanding aquatic–terrestrial linkages. *Molecular Ecology*, **23**, 5888–5903.

Crutsinger G.M., Rudman S.M., Rodriguez-Cabal M.A., Mckown A.D., Sato T., Macdonald A.M. *et al.* (2014b) Data from: testing a "genes-to-ecosystems" approach to understanding aquatic–terrestrial linkages. *Dryad Digital Repository*. doi:10.5061/dryad.1nq6m.

Farrar J. (1995) *Trees in Canada*. Fitzhenry & Whiteside Ltd., Markham, and Canadian Forest Service, Natural Resources Canada, Ottawa.

Fisher R.A. (1930) *The Genetical Theory of Natural Selection: A Complete Variorum Edition*. Oxford University Press, Oxford.

Geraldes A., Difazio S., Slavov G., Ranjan P., Muchero W., Hannemann J. *et al.* (2013) A 34K SNP genotyping array for *Populus trichocarpa*: design, application to the study of natural populations and transferability to other *Populus* species. *Molecular Ecology Resources*, **13**, 306–323.

Geraldes A., Farzaneh N., Grassa C.J., McKown A.D., Guy R.D., Mansfield S.D. *et al.* (2014) Landscape genomics of *Populus trichocarpa*: the role of hybridization, limited gene flow and natural selection in shaping patterns of population structure. *Evolution*, **68**, 3260–3280.

Gessner M.O., Swan C.M., Dang C.K., Mckie B.G., Bardgett R.D., Wall D.H. *et al.* (2010) Diversity meets decomposition. *Trends in Ecology & Evolution*, **25**, 372–380.

Harper M.P. & Peckarsky B.L. (2006) Emergence cues of a mayfly in a high-altitude stream ecosystem: potential response to climate change. *Ecological Applications*, **16**, 612–621.

Hieber M. & Gessner M.O. (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026–1038.

Holt R.D. (2008) Theoretical perspectives on resource pulses. *Ecology*, **89**, 671–681.

Hughes A.R., Inouye B.D., Johnson M.T., Underwood N. & Vellend M. (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.

Hughes A.R. & Stachowicz J.J. (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 8998–9002.

Jackrel S.L. & Wootton J.T. (2015) Cascading effects of induced terrestrial plant defences on aquatic and terrestrial ecosystem function. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20142522.

Johansson J. & Jonzen N. (2012) Effects of territory competition and climate change on timing of arrival to breeding grounds: a game-theory approach. *The American Naturalist*, **179**, 463–474.

Johnson M.T. & Stinchcombe J.R. (2007) An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology & Evolution*, **22**, 250–257.

Leroux S.J. & Loreau M. (2012) Dynamics of reciprocal pulsed subsidies in local and meta-ecosystems. *Ecosystems*, **15**, 48–59.

LeRoy C.J., Whitham T.G., Keim P. & Marks J.C. (2006) Plant genes link forests and streams. *Ecology*, **87**, 255–261.

LeRoy C.J., Whitham T.G., Wooley S.C. & Marks J.C. (2007) Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. *Journal of the North American Benthological Society*, **26**, 426–438.

Lindroth R.L. & Hwang S.-Y. (1996) Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). *Biochemical Systematics and Ecology*, **24**, 357–364.

Madritch M., Donaldson J.R. & Lindroth R.L. (2006) Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems*, **9**, 528–537.

Madritch M.D. & Hunter M.D. (2002) Phenotypic diversity influences ecosystem functioning in an oak sandhills community. *Ecology*, **83**, 2084–2090.

Madritch M.D. & Lindroth R.L. (2011) Soil microbial communities adapt to genetic variation in leaf litter inputs. *Oikos*, **120**, 1696–1704.

Marcarelli A.M., Baxter C.V., Mineau M.M. & Hall R.O. Jr (2011) Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology*, **92**, 1215–1225.

McKown A.D., Guy R.D., Azam M.S., Drewes E.C. & Quamme L.K. (2013) Seasonality and phenology alter functional leaf traits. *Oecologia*, **172**, 653–665.

McKown A.D., Guy R.D., Klápště J., Geraldes A., Friedmann M., Cronk Q.C.B. *et al.* (2014a) Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. *New Phytologist*, **201**, 1263–1276.

McKown A.D., Guy R.D., Quamme L., Klápště J., La Mantia J., Constabel C.P. *et al.* (2014c) Association genetics, geography and ecophysiology link stomatal patterning in *Populus trichocarpa* with carbon gain and disease resistance trade-offs. *Molecular Ecology*, **23**, 5771–5790.

McKown A.D., Klápště J., Guy R.D., Geraldes A., Porth I., Hannemann J. *et al.* (2014b) Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of *Populus trichocarpa*. *New Phytologist*, **203**, 535–553.

McNamara J.M., Barta Z., Klaassen M. & Bauer S. (2011) Cues and the optimal timing of activities under environmental changes. *Ecology Letters*, **14**, 1183–1190.

Moore J.W. & Schindler D.E. (2010) Spawning salmon and the phenology of emergence in stream insects. *Proceedings of the Royal Society of London B: Biological Sciences*, **277**, 1695–1703.

Morisette J.T., Richardson A.D., Knapp A.K., Fisher J.I., Graham E.A., Abatzoglou J. *et al.* (2009) Tracking the rhythm of the seasons in the face of global change: phenological research in the 21st century. *Frontiers in Ecology and the Environment*, **7**, 253–260.

Murphy J. & Riley J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.

Polgar C.A. & Primack R.B. (2011) Leaf-out phenology of temperate woody plants: from trees to ecosystems. *New Phytologist*, **191**, 926–941.

Porter L.J., Hrstich L.N. & Chan B.C. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**, 223–230.

Primack R.B. (1980) Variation in the phenology of natural populations of montane shrubs in New Zealand. *The Journal of Ecology*, **68**, 849–862.

Reed T.E., Gienapp P. & Visser M.E. (2015) Density dependence and microevolution interactively determine effects of phenology mismatch on population dynamics. *Oikos*, **124**, 81–91.

Revilla T.A., Encinas-Viso F. & Loreau M. (2014) (A bit) Earlier or later is always better: phenological shifts in consumer–resource interactions. *Theoretical Ecology*, **7**, 149–162.

Rudman S.M., Rodriguez-Cabal M.A., Stier A., Sato T., Heavyside J., El-Sabaawi R.W. *et al.* (2015) Adaptive genetic variation mediates bottom-up and top-down control in an aquatic ecosystem. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20151234. doi:10.1098/rspb.2015.1234.

Schweitzer J.A., Bailey J.K., Hart S.C. & Whitham T.G. (2005) Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology*, **86**, 2834–2840.

Slavov G.T., Difazio S.P., Martin J., Schackwitz W., Muchero W., Rodgers-Melnick E. *et al.* (2012) Genome resequencing reveals multiscale geographic structure and extensive linkage disequilibrium in the forest tree *Populus trichocarpa*. *New Phytologist*, **196**, 713–725.

Stachowicz J.J., Kamel S.J., Hughes A.R. & Grosberg R.K. (2013) Genetic relatedness influences plant biomass accumulation in eelgrass (*Zostera marina*). *The American Naturalist*, **181**, 715–724.

Takimoto G., Iwata T. & Murakami M. (2002) Seasonal subsidy stabilizes food web dynamics: balance in a heterogeneous landscape. *Ecological Research*, **17**, 433–439.

Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science*, **277**, 102–104.

Weber J., Stettler R. & Heilman P. (1985) Genetic variation and productivity of *Populus trichocarpa* and its hybrids. I. Morphology and phenology of 50 native clones. *Canadian Journal of Forest Research*, **15**, 376–383.

Wegrzyn J.L., Eckert A.J., Choi M., Lee J.M., Stanton B.J., Sykes R. *et al.* (2010) Association genetics of traits controlling lignin and cellulose biosynthesis in black cottonwood (*Populus trichocarpa*, Salicaceae) secondary xylem. *New Phytologist*, **188**, 515–532.

Whitham T.G., Bailey J.K., Schweitzer J.A., Shuster S.M., Bangert R.K., Leroy C.J. *et al.* (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics*, **7**, 510–523.

Whitham T.G., Gehring C.A., Lamit L.J., Wojtowicz T., Evans L.M., Keith A.R. *et al.* (2012) Community specificity: life and afterlife effects of genes. *Trends in Plant Science*, **17**, 271–281.

Xie C.-Y., Carlson M.R. & Ying C.C. (2012) Ecotypic mode of regional differentiation of black cottonwood (*Populus trichocarpa*) due to restricted gene migration: further evidence from a field test on the northern coast of British Columbia. *Canadian Journal of Forest Research*, **42**, 400–405.

Yang L.H., Bastow J.L., Spence K.O. & Wright A.N. (2008) What can we learn from resource pulses. *Ecology*, **89**, 621–634.

Yang L.H. & Rudolf V. (2010) Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecology Letters*, **13**, 1–10.

(Manuscript accepted 18 October 2016)