Effects of native and invasive alien decapods on freshwater biodiversity and ecosystem functioning

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Abstract

Invasive alien species threaten freshwater biodiversity, with uncertain impacts on ecosystem services. Invasive alien decapods are frequent components of invaded ecosystems, and have been found to impact biodiversity, ecosystem properties and processes. Studies however often do not consider comparisons to native analogues. Native analogues could mask impacts of invasive alien species through functional redundancy. The native White Clawed Crayfish (*Austropotamobius pallipes*) is declining at the advance of the invasive alien American Signal Crayfish (*Pacifastacus leniusculus*) and the Chinese Mitten Crab (*Eriocheir sinensis*). Consequences of this changeover of decapod species to the patterns and processes of river basins are not well understood.

Laboratory microcosm experiments found invasive alien decapods processed native leaf litter at a greater rate than native crayfish, increasing smaller fragments, notably fine particulate organic matter and dissolved organic carbon (Chapter 2). However, these differences in transformation of leaf litter into other products did not increase the productivity of algal biofilms. Each decapod species excreted nutrients at different rates: *A. pallipes* and *E. sinensis* had similar nitrogen excretion, while *P. leniusculus* excreted much less nitrogen suggesting this species could be a nutrient sink. These differences in nutrients were apparently absorbed by biofilms growing on leaf and rock surfaces even though they did not grow as a result.

Chapter 3 found the greater detritivory performance of invasive alien decapods was observed for other species of leaf litter, including increased breakdown of particularly troublesome invasive alien riparian plant *Rhododendron ponticum*. Derived products varied, with more fine particulate organic matter generated for *Acer pseudoplatanus* and *R. ponticum*, suggesting less assimilation

of these litter types. This revealed a previously unknown feedback between invasive plants and detritivores, whereby the litter accumulations from riparian invasive plants is potentially consumed by invasive alien decapods. This in turn may subsidise the growth and reproduction of invasive alien aquatic decapods.

Outdoor pond mesocosm experiments revealed that invasive alien decapods altered taxonomic richness, diversity, and structure, with snails particularly found to decline in ponds (Chapter 4). Primary productivity of periphyton was elevated in *P. leniusculus* ponds compared to natives, but not in the *E. sinensis* ponds, explained by a combination of trophic cascade and turbidity. Leaf litter decomposition rates, community respiration, gross and net primary productivity were otherwise unaffected. Outdoor flume mesocosms were utilised to replicate headwater streams to measure bioturbation of decapods, and associated changes to biodiversity, water quality and ecosystem processes (Chapter 5). Effects on sediment topology and transport were similar for controls, native and alien species, with the presence of refuges likely causing this lack of effect. Suspended organic matter was similar for all species, but sediment respiration was higher for *P. leniusculus*. Invertebrate community structure was significantly different for invasive alien decapods, with significantly higher densities of collector-gathering invertebrates such as *Baetis* sp. and Culicoides.

The the lack of consensus between the laboratory (Chapters 2-3) and mesocosm studies (Chapters 4-5) lead to the conclusion that environmental practitioners should consider context and scaling when interpreting experimental results of biological invasions. The overall findings of this study however do show that *P. leniusculus* and *E. sinensis* are not functionally redundant for several ecosystem attributes compared to *A. pallipes*. These differences in species functional-traits are likely to be exacerbated by greater densities of invasives observed *in situ*. This supports the potential for invasive alien decapods to alter the ecosystem services of entire river basins, even when a native analogue was previously present.

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General Introduction

i. Thesis introduction

Biological invasions are a well-known global phenomenon arising from the migration and development of human civilization (Lowe et al., 2000, Simberloff et al., 2012a, Simberloff, 2013). They were initially reported in the proliferation of novel agricultural pests (Elton, 1958), and the extirpation of island species caused by non-native species translocated by humans (Sax et al., 2002). The advent of research on the impact of biodiversity on ecosystem functioning in the 1990s (onwards from Vitousek and Hooper, 1993) has led to research on the consequences of invasive alien species for whole ecosystems. This moves invasion biology from pathways of introduction, invasion success, and interactions with other species, to changes to whole species assemblages, biogeochemical processes and ecosystem services. These whole ecosystem responses are particularly important to study in freshwater ecosystems given their importance for society.

This thesis examines the ecosystem consequences of one invasion syndrome (*sensu* Kueffer et al., 2013) in European freshwaters: the invasion of the American Signal Crayfish (*Pacifastacus leniusculus* Dana, 1852) and Chinese Mitten Crab (*Eriocheir sinensis* H. Milne-Edwards, 1853) that is replacing the previously widespread White Clawed Crayfish (*Austropotamobius pallipes* Lereboullet, 1858). Chapter 1 reviews the available evidence so far obtained by other researchers on the ecological outcomes of this invasion syndrome, with research gaps identified. The subsequent chapters use lab and mesocosm approaches and examine the impacts of these three species on: detrital processing in a lab study (Chapter 2); processing of detritus from native and invasive leaf litter (Chapter 3); multi-trophic species assemblages and their ecosystem processes in outdoor lentic mesocosms (Chapter 4); and ecosystem engineering effects in outdoor flume mesocosms (Chapter 5). The final chapter (6) synthesises these studies, exploring the challenge of assessing and predicting the consequences of biological invasions from experimental venues of differing scale and context.

Chapter 1. Invasions of alien decapods into the realms of a native analogue: Consequences of maintenance of species richness but different species composition

1.1 Literature Review

1.1.1 Invasive alien species: definitions, impacts and the current need for research

Invasive alien species are organisms translocated anthropogenically from their native range to a biogeographically novel locality, where they become established and spread (Blackburn et al., 2011). These pathways for introduction can either be intentional such as the introduction of a new crop, or accidental such as the attachment of propagules to an agent of novel transport. This terminology differs from the term “invasion” applied to succession ecology, in which the biogeographic origin of a species colonizing (invading) a patch is not necessarily considered (Young et al., 2001). This has linguistically caused confusion among biologists and the public, especially when certain governing bodies identify native species as “invasive” when they become problematic to society (Lodge and Shrader-Frechette, 2003, Simberloff et al., 2012b). Traits of both native and non-native invasive species frequently consist of high fecundity and colonization, but non-native invasive species may also be released from biological interactions with other species and conditions that regulate their populations in their home range (Colautti et al., 2004). They can therefore have different impacts to native “invasives” (Simberloff et al., 2012b). Hence, the term “alien” is often applied to species with no natural biogeographic history where it occurs, and will hereafter be referred to as invasive alien species, terminology also used by the European Union for their management (Genovesi and Shine, 2004). Other synonyms can include “alien”, “exotic”, “non-indigenous”, “imported”, “introduced”, “non-native” (Valéry et al., 2008, Blackburn et al., 2011).

Non-native species are usually considered “invasive” if they have been shown to impact their recipient ecosystems (Parker et al., 1999, Valéry et al., 2008). Negative impacts of invasive alien species observed so far include inducing declines and extirpation of native species through either their consumption, competition or hybridization (Simberloff, 2013, Bellard et al., 2016). An invasive alien species might also co-introduce other harmful species to native assemblages, such as pathogens (Simberloff and Von Holle, 1999, Dunn and Hatcher, 2015, Roy et al., 2016). Invasive alien species have been attributed to local and global declines and extinctions of numerous species, particularly on islands (Sax et al., 2002, Bellard et al., 2016), even after positive impacts on certain attributes such as facilitating certain native species have been considered (Rodriguez, 2006, Schlaepfer et al., 2011). Globally, invasive alien species are associated with a homogenized pan-global biota, particularly in and adjacent to areas of regular human activity (McKinney and Lockwood, 1999, Toussaint et al., 2016). Impacts of this homogenization on ecosystem processes are not fully understood, though these “novel” communities of multiple non-native species are predicted to provide at least some ecosystem services such as nutrient cycling, pest control and food resources (Hobbs et al., 2006). Certain invasive alien species can also physically alter the environment of an ecosystem, with cascading effects on the biota (Fei et al., 2014). For example, invasive alien bivalves increase clarity of the water column, increasing biomass of benthic organisms (Sousa et al., 2009). These changes to the biotic and abiotic environment could therefore lead to changes in the flow of energy and biogeochemical cycling in the ecosystem, as well as general ecosystem properties (Strayer, 2012).

The impact of an invasive alien species often only becomes evident once the invasive species has achieved high densities and broad ranges (Parker et al., 1999, Blackburn et al., 2014). Inferences of impact are therefore based on the success of invasion, notably the rate of proliferation, spread and achievement of dominance (Parker et al., 1999). Impacts of a particular alien species are also predicted based on impacts in other localities, but environmental conditions of different recipient ecosystems can result in unpredictable invasion success and impact (Kolar and Lodge, 2001). These uncertainties therefore necessitate the approach of “guilty until proven innocent” by environmental practitioners, which advocates the pre-emptive action of preventing ongoing and new introductions and managing populations of introduced alien species (Vitule et al., 2009). It is however important to explore the impacts of invasive species in order to understand and predict invasion impact and to target resources towards control and biosecurity.

Controlled experiments and rigorous field observations on recipient ecosystems are therefore necessary to verify causal effects of invasive alien species on ecosystems (Blackburn et al., 2010, Strayer, 2012). Techniques to assess the ecological impacts of non-native species involve experiments at different scales ranging from the laboratory studies often looking at interactions of two species to more complex mesocosms exploring community effects to *in-situ* enclosures to observations of ongoing invasions (of *in-situ* natural experiments) where invasion history is known and stratified. Controlled and highly replicated experiments undertaken in laboratory microcosms often suffer from a lack of realism which might affect the upscaling of results to the real world (Schindler, 1998). However, directly measuring response variables in an ecosystem associated with an invasive alien species' arrival maximises realism at the expense of replication, and can result in spurious inferences about causality if other changes are also occurring, such as habitat modification (MacDougall and Turkington, 2005) or changing climate (Daszak et al., 2005). The use of *in-situ* enclosures experimentation depends on the use of already invaded habitats that could be under the influence of "the ghost of invasion past" (Kueffer et al., 2013). Studies on the effects of invasive alien species therefore require controlled experimental treatments to minimise or remove such confounding effects. A middle ground is the use of mesocosm studies in realistic outdoor settings, which standardise variation of environmental conditions (temperature, day length, exposure) (Harris et al., 2007, Ledger et al., 2009, Benton et al., 2007) and which also allow improved levels of replication relative to field studies (Schindler, 1998).

1.1.2 Impacts of invasive alien species on freshwater ecosystems

Freshwater ecosystems have been impacted particularly by invasive alien species in many parts of the world (Strayer and Dudgeon, 2010, Moorhouse and Macdonald, 2015). Freshwater invasive alien species typically consist of species that have arrived accidentally e.g. through ballast water in ships, non-intentionally such as release of bait species or deliberately introduced (such as for food), escapes from the pet or garden trade, or are introduced intentionally (Keller et al., 2009). Freshwater ecosystems with long term introductions of non-native species can become dominated by invasive alien species: for example, 90% of the River Rhine's invertebrate species are invasive alien species (Van Riel et al., 2006). Freshwater invasive alien species include species from diverse taxa and functional traits. For example, 141 invasive alien species are represented in the freshwater ecosystems of Great Britain, and consist of primary producers, collector-gatherers, shredders, filter-feeders, predators and combinations of these (Table 1.1).

The impacts of invasive alien species on freshwater ecosystems are broad and increasingly better understood, and appear to be linked to the functional traits of the species (Fig 1) (Strayer, 2010, Thomsen et al., 2014, Gallardo et al., 2015). Invasive alien species can impact biotic properties that include diversity and population size of various native species at various trophic levels and their biodiversity. These impacts are mediated by trophic, competitive or habitat-invader interactions (Gallardo et al., 2015). Abiotic aspects of freshwater ecosystems that can be affected by invasive alien species include physical properties such as geomorphology, flow, turbidity (Fei et al., 2014). The abiotic and biotic changes caused by invasive alien species can lead to alterations of ecosystem functioning, including decomposition rates, primary productivity and nutrient cycling (Strayer, 2012, Gallardo et al., 2015). These impacts on ecosystems are however still not fully understood, especially in the contexts of replacement by an invasive alien species of an analogue native species, and in the absence confounding or synergising factors such as habitat type or disturbance (Strayer, 2012).

Table 1.1. Summary of freshwater invasive alien species on the island of Great Britain by taxonomic group and functional guild (from Keller et al., 2009, MacNeil et al., 2010, Aldridge et al., 2014, information on guilds derived from Usseglio-Polatera et al., 2000).

Taxonomic Group	Guilds represented	Number of species
Virus	Parasite	1
Plants (aquatic)	Primary Producer: marginal, floating, submerged	31
Plants (riparian)	Primary Producer: riparian	4
Fungi	Parasite	2
Cnidarians	Filter-feeder; Predator; Zooplankton	2
Nematodes	Parasite	2
Platyhelminthes	Parasite	15
Oligochaeta	Collector-gatherer	5
Mollusca (Bivalvia)	Filter-feeder	5
Mollusca (Gastropoda)	Grazer	6
Insecta	Grazer	1
Crustaceans (Maxillopoda)	Parasite	10
Crustaceans (Cladocera)	Zooplankton	1
Crustaceans (Isopoda)	Collector-gatherer; Shredder	1
Crustaceans (Mysida)	Collector-gatherer	1
Crustaceans (Amphipoda)	Shredder; Collector-gatherer; Predator	6
Crustaceans (Decapoda)	Shredder; Collector-gatherer; Predator	7
Fishes	Grazer; Predator	18
Amphibia (Caudata)	Predator	3
Amphibia (Anura)	Collector-gatherer; Grazer; Predator	8
Birds	Grazer	8
Mammals	Grazer; Predator	4

Invasive alien primary producers, such as marginal, floating, submerged and riparian plants can out-compete other plant species, and shade out water bodies, reducing light penetration and gross primary productivity of those water bodies (Hladyz et al., 2011, Gallardo et al., 2015). These invasive alien plants can also accumulate novel leaf litter in water bodies that may be unsuitable for consumption by detritivorous species, and thus reduce secondary productivity (Hladyz et al., 2009, Hladyz et al., 2011). Invasive alien filter-feeders can reduce the biomass of both phytoplankton and zooplankton, and increase light penetration resulting in greater productivity of benthic primary producers and invertebrates (Strayer et al., 1999, Sousa et al., 2009, Gallardo et al., 2015). Invasive alien grazer species, notably snails, have been found to reduce benthic primary producer biomass, and increase phytoplankton production through enriching the water column with nutrients (Carlsson et al., 2004), with grazing fish species also increasing turbidity through disturbance of sediment (Matsuzaki et al., 2007). Many invasive alien consumers are omnivores that have detrital shredding traits (*sensu* Cummins 1973) and are also collector gatherers and/or predators (MacNeil et al., 1997, Gallardo et al., 2015). Invasive alien omnivores have been found to be either more predatory than detritivorous, resulting in reduced decomposition rates compared to native analogues (MacNeil et al., 2011, Piscart et al., 2011) or process to detritus at an increased or equivalent rate (Dunoyer et al., 2014, Lagrue et al., 2014).

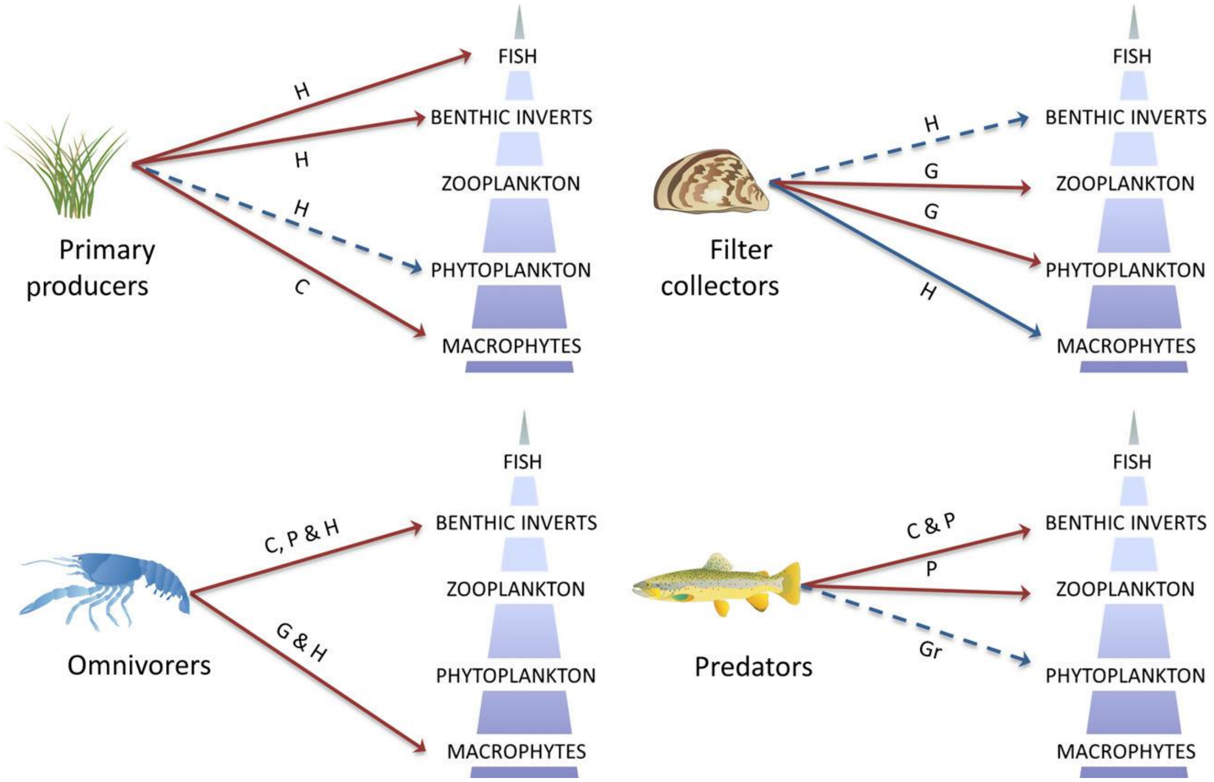

Figure 1.1. Impacts of different functional guilds of freshwater invasive alien species on different levels of a food web. From Gallardo et al., 2015: Arrows reflect the negative (unbroken arrows) or positive (dashed arrows) impacts of invasive species on the abundance of five different functional components of resident communities. Impacts are the result of a combination of direct ecological (C, competition, P, predation, G, grazing) and indirect physicochemical impacts of invasive species (Gr, grazer release, H, habitat alteration).

An ecosystem that accumulates species is predicted to show an increase in traits that will either be complementary or redundant among species (Walker, 1992, Naeem, 1998). However, many studies on the impacts of invasive alien species have not considered redundancy with regard to the native species assemblage, instead measuring the impacts without an analogue species present (Dunoyer et al., 2014). In many cases, a taxonomically similar and functionally analogous native species might not be present, which has been attributed to the cause of dramatic impacts by invasive alien species (Ricciardi and Atkinson, 2004, Paolucci et al., 2013). An invasive alien species that has similar ecological impacts to a native species that it might out-compete could therefore have negligible impacts on a recipient ecosystem. However, if an non-native species has different ecological impacts to a similar native species, then it would be predicted to have negative impacts through destabilizing an ecosystem (Magoulick and Piercey, 2016).

1.1.3 Invasive alien decapods in freshwater ecosystems

Some of the most prominent and impactful invasive alien species in freshwaters are omnivorous decapod Crustacea, which are conspicuous in many freshwaters subjected to general biological invasions (Lodge et al., 2012). This is likely a consequence of their considerable effects on freshwater ecosystems in their native ranges (Lodge et al., 1994, Parkyn et al., 1997, Usio, 2000, Usio and Townsend, 2002, Dorn and Wojdak, 2004, Rosewarne et al., 2013, Zhang et al., 2004). Because decapods are omnivorous, they can influence several trophic levels: leaf litter (Emmerson and McGwynne, 1992, Usio, 2000, Schofield et al., 2008, Dunoyer et al., 2014); carrion (Gladman et al., 2012); living macrophytes (Nyström and Strand, 2003); algal biofilms (Gherardi and Lazzara, 2006); invertebrate consumers (Nyström et al., 1999, Gherardi et al., 2001, Nyström et al., 2001, Stenroth and Nyström, 2003, Crawford et al., 2006, Peay et al., 2009, Haddaway et al., 2012, Rosewarne et al., 2013, Arribas et al., 2014); fish (Guan and Wiles, 1997, Peay et al., 2009); and amphibians (Gherardi et al., 2001). These invaders also have the potential for altering sediment erosion and transport in rivers (Statzner et al., 2000, Harvey et al., 2011, Johnson et al., 2011) and structure of riparian zones through burrowing (Holdich et al., 1999, Guan, 2010, Harvey et al., 2011). These combined impacts on biotic and abiotic ecosystem variables therefore have the potential to affect freshwater ecosystem functioning. For example, trophic impacts can result in cascades leading to increased periphyton production (Nyström et al., 2001, Stenroth and Nyström, 2003, Jackson et al., 2014, Rosewarne et al., 2016) and decreased detritus decomposition (Dunoyer et al., 2014, Lagrue et al., 2014). Effects of increased sediment flux into the water column or alteration of habitat structure by decapods on ecosystem processes have not been explicitly researched, though increased fine sediment concentration in the water column does impact sediment-sensitive species (Jones et al., 2012). Crayfish habitat modification in mesocosms have also been associated with decreased algae cover (Statzner et al., 2000, Statzner and Sagnes, 2008) but it was not clear whether this was due to consumption or engineering by crayfish.

Research on invasive alien decapods has typically been in the context of the decapod becoming introduced to ecosystems naïve to this taxonomic group (e.g. Crawford et al. 2006; Grey & Jackson, 2012; Moore et al. 2012, James et al. 2014). In contrast the ecological consequences of replacement of native decapods species by non-native species have been poorly studied. Two meta-analyses of the impacts of crayfish on biodiversity and ecosystem functioning only compared impacts of crayfish upon crayfish-free controls and not to treatments with analogous natives (Gallardo et al., 2015, James et al., 2015). One meta-analysis was able to make use of eight laboratory and enclosure experiments comparing native to invasive alien crayfish, but the majority were in North America, and were limited to measurements of single taxa or trophic levels (algae, macrophytes, snails, other invertebrates, fish or amphibians, Twardochelb et al., 2013) rather than assessing impacts across multiple trophic levels, ecosystem attributes and processes. Thus this thesis provides novel information on the responses of multiple ecosystem variables to the replacement of native by invasive alien decapods through factorial experiments.

1.1.4 The decline of the White Clawed Crayfish and its replacement by alien decapods

The White Clawed Crayfish (*Austropotamobius pallipes* Lereboullet, 1858) is one of three species of its genus, and was historically common in northern Europe west of the Alps, particularly in headwater streams, but also in lakes and larger river basins (Souty-Grosset et al., 2006). This species has become extirpated across much of its former range in Europe as a result of competition from invasive alien crayfish species which were originally introduced for food, which subsequently escaped or were released from crayfish farms. *Austropotamobius pallipes* is extinct throughout most of southern Great Britain, but there are populations in Wales, Cumbria, Northumberland, North Yorkshire and West Yorkshire (Figure 1.2). *Austropotamobius pallipes* has recently been classified as Endangered by the IUCN Redlist (Füreder, 2010). The rate of decline in range per country ranges from 50-80% within 10 years, with extinction of this species predicted without intervention (Sibley et al., 2002, Füreder, 2010) particularly in Great Britain (Fig 1.2). The American Signal Crayfish (*Pacifastacus leniusculus* Dana 1852) was introduced from the Pacific Northwest of America is the invasive alien decapod most frequently implicated in *A. pallipes* decline (Sibley et al., 2002), though other invasive decapods that have an impact include the Turkish Crayfish (*Astacus leptodactylus* Eschscholtz 1823) and the Red Swamp Crayfish (*Procambarus clarkii* Girard 1852) (Holdich et al., 2009). These invasive alien crayfish have caused extirpations of *A. pallipes* through competition for refugia that makes the native crayfish more vulnerable to predation (Bubb et al., 2006), with the spread of an invasive alien pathogen, the Crayfish Plague Fungus *Aphanomyces astaci*, by the invasive alien crayfish has been a notable factor causing the rapid decline of *A. pallipes* (Alderman et al., 1990, Holdich and Reeve, 1991). Even in the absence of disease, *P. leniusculus* has been shown to extirpate *A. pallipes*, such as on the River Wharfe in England (Dunn et al., 2009, Imhoff et al., 2011).

Another invasive alien decapod is the Chinese Mitten Crab (*Eriocheir sinensis* H. Milne-Edwards 1853). This invasive alien species has become introduced through accidental releases of ballast water and has spread from its native range in south-east Asia to Europe and North

America (Clark et al., 1998, Herborg et al., 2007). This species is catadromous: it breeds in saltwater, but migrates upriver to freshwater habitats after development as larvae, where they spend up to 5 years maturing (Veilleux and De Lafontaine, 2007). Their breeding migrations are thought to substantially affect biomass transport from upstream (Lowe et al., 2000), and other impacts include creating very dense burrows in river banks (Dutton and Conroy, 1998, Rudnick et al., 2005) and consumption of detritus, plants, invertebrates and fish eggs (Rudnick and Resh, 2005, Webster et al., 2015, Rosewarne et al., 2016). *Eriocheir sinensis* has been shown to out-compete native shoreline crabs for shelter (Gilbey et al., 2008). However, its impact on native freshwater decapods has not been explored. Clark et al. (1998) predicted *E. sinensis* would have a potentially detrimental impact on *A. pallipes* as with other invasive alien decapods, and has recently been found to be a carrier of Crayfish Plague Fungus (Schrimpf et al., 2014). It is also possible that *E. sinensis* will out-compete the widespread invasive *P. leniusculus*. In order to explore potential impact of replacement of native and invasive crayfish by *E. sinensis*, this study directly compares the impact of *E. sinensis*, *P. leniusculus* and *A. pallipes* on invertebrate density, biodiversity, ecosystem properties and processes.

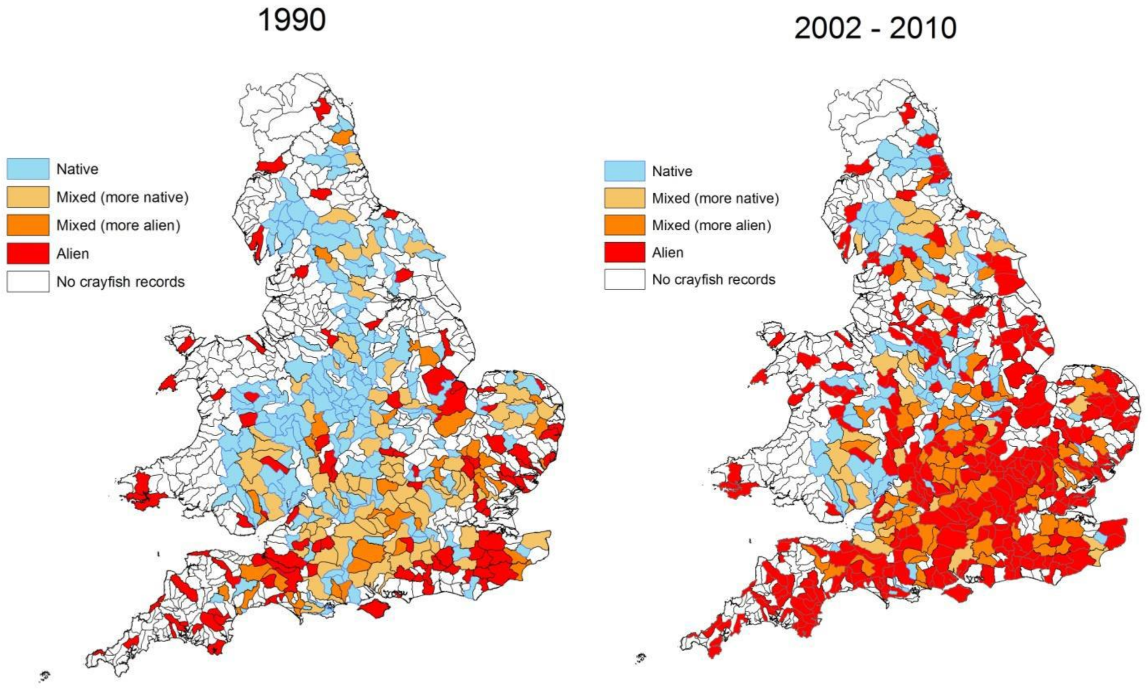

Figure 1.2. Changing range of native (*Austropotamobius pallipes*) at the advance of invasive alien decapods (primarily *Pacifastacus leniusculus*) in Great Britain (Rogers and Watson, 2010)

1.1.5 Potential impacts of turnover of native to invasive alien decapods

To understand the impacts of the replacement of *A. pallipes* by *P. leniusculus* and *E. sinensis*, known ecological effects of these species are here reviewed. All species are omnivorous, consuming detritus, macrophytes, benthic invertebrates, carrion, eggs of vertebrates and vertebrates smaller than themselves. *A. pallipes* has been found to regulate benthic invertebrate populations and their derived functional impacts (such as grazing of periphyton), as observed during one disease-induced population decline (Matthews and Reynolds, 1992), through replicated mesocosm (Rosewarne et al., 2013) and laboratory studies (Haddaway et al., 2012). Similarly, *P. leniusculus* and *E. sinensis* have both been found to impact lower trophic levels, depleting detritus and abundance of invertebrates in both laboratory, cage and mesocosm experiments, in addition to field observations (Rudnick and Resh, 2005, Haddaway et al., 2012, Moore et al., 2012, Lagrue et al., 2014, Rosewarne et al., 2016). Differences in impacts between these three decapod species are therefore best predicted based on species-specific consumption rates of various trophic resources.

If size-matched individuals of each species have the same impact on various attributes of an ecosystem, then the invasion could be predicted to be functionally redundant (Magoulick and Piercey, 2016) and no impact would be predicted (*sensu* Parker et al. (1999). This is before accounting for between-species differences in body size, population growth rate and density of individuals that can mechanistically predict impacts. Differences in the body size and density of these species are given in Table 1.2. The two invasive alien decapod species typically achieve greater size, fecundity, growth rate and densities compared to A. pallipes. In this thesis, per capita effects of these decapod species are explored, which in combination with data on density and size, can inform predictions of impact in the field (Parker et al., 1999).

Table 1.2. Summary of traits of native and invasive alien decapod crustaceans in the study

Species	**Native range**	**Introduced range**	**Maximum length***	**Fecundity (eggs per female)**	**Maximum density achieved (individuals** m^{-2}**)**	**Source**
Austropotamobius pallipes	Europe west of the Alps	Ireland (though debated)	12 cm	20-160	4.8	Nyström, 2002
Pacifastacus leniusculus	North-western North America	Elsewhere in North America, Europe, Japan	16 cm	200-500	15	Nyström, 2002
Eriocheir sinensis	China	North America, Europe	10 cm	250,000-1,000,000	19	Rudnik et al., 2003

* Crayfish length is from the rostrum to the telsom, while crab length is based on carapace width.

Laboratory experiments have so far shown *P. leniusculus* and *E. sinensis* have a greater consumption rate of prey than *A. pallipes* (Haddaway et al., 2012, Rosewarne et al., 2016). *Pacifastacus leniusculus* has also been found to consume fish eggs and larvae (Edmonds et al., 2011). The scaling-up of these results to the real world scenarios, such as with mesocosms, has however not been assessed. While these species have been studied in mesocosms, they have so far not been studied in a factorial experiment simultaneously. Their impacts on ecosystem functioning has also not been studied comprehensively, with the differences in predatory functional responses on snails potentially translating into stronger trophic cascades on primary productivity of periphyton and macrophytes. The larger body size of the invasive alien decapods could also induce stronger impacts on sediment transport, which could impact penetration of light to the benthos. Sequentially, this could impact gross primary productivity of the ecosystem.

1.2 Thesis aim and outline

The aim of this thesis is to assess the ecological impacts of the extinction of *A. pallipes* and the consequential (as well as causative) invasion and establishment of *P. leniusculus* and *E. sinensis*. The recurrent approach is to compare the impacts of size-matched individuals of each species to evaluate *per capita* impacts and assess whether the impacts are redundant or novel; and then to scale up to more realistic mesocosms to compare effects on ecosystem processes. As laboratory studies have already established that the invasive alien species show novel consumptive traits for invertebrate prey, the overall thesis hypothesises that this would also occur for other resources, and be observed in more realistic experimental venues. These enhanced functional responses of the invasive alien species suggest that invertebrates and other resources such as leaf litter and macrophytes would be depleted in relation to the native, resulting in altered ecosystem functioning. The increased foraging of the invasive alien species and reported burrowing behaviour is also expected to undergo an engineering effect on sediments, consequently altering turbidity of water and sediment topology.

Chapter 2 examines the impacts of the three focal decapod crustaceans on detrital processing, where a basal trophic resource is converted to other products. In this controlled laboratory study, the potential influence on detrital processing, biofilm growth and photosynthesis was assessed. Upon discovering differential detrital processes between the native and invasive alien decapod species, Chapter 3 measured the efficacy of invasive alien plant litter processing by these decapods. Chapter 4 uses outdoor mesocosms to assess if observations on the functional responses of different decapod species measured in simplified laboratories could predict impacts on biodiversity and ecosystem functioning in more realistic, biocomplex venues. This included measurements of invertebrate density, diversity, water quality, decomposition rates, biofilms, macrophyte biomass, community respiration and gross primary productivity. Chapter 5 measured the ecosystem engineering impacts of the native and invasive alien decapods on sediments of flume mesocosms. This experiment also took the opportunity to examine the impacts of the decapods on biodiversity and ecosystem processes similar to the work undertaken in Chapter 4. The final chapter synthesises the studies conducted, assessing the consensus between them and the cost-benefits of utilizing these different experimental venues for assessing the impacts of the replacement of native crayfish by these two prominent invasive alien decapod species.

Chapter 2. Decomposition of leaf litter by a European native and two invasive alien freshwater decapods

2.1 Introduction

Invasive alien species can alter community structure and modify ecosystem processes (Lafferty et al., 2006, Ehrenfeld, 2010, Simberloff et al., 2012a, Strayer, 2012, Dunn et al., 2012), especially in freshwater ecosystems (Strayer and Dudgeon, 2010, Moorhouse and Macdonald, 2015). One major freshwater ecosystem process is the recycling of leaf litter and its transformation into other forms of energy and nutrient throughout the food web (Cummins et al., 1973, Vannote et al., 1980, Gessner et al., 2010). A recent global meta-analyses of the impacts of aquatic invasive species on lower trophic levels and ecosystem attributes found substantial impacts, including on nitrogen and organic matter standing stocks but not explicitly examining detritus decomposition rates (Gallardo et al., 2015). Most studies on the impacts of invasive alien species on leaf litter decomposition have focused predominately on leaf litter from invasive alien riparian plants (Lecerf et al., 2007, Godoy et al., 2010, Hladyz et al., 2011). Studies on invasive alien consumers of leaf litter have been fewer, although some noteworthy studies on macroinvertebrate shredders such as amphipod species (*Gammarus tigrinus* and *Dikerogammarus villosus*) showed reduced decomposition rates in the presence of more predatory (invasive alien) species (MacNeil et al., 2011, Piscart et al., 2011). Freshwater decapods, particularly crayfish, are key processors of allochthonous riparian resources in their native ranges, with crabs studied to a lesser extent (Kobayashi et al., 2011, Rosewarne et al., 2013, Schofield et al., 2001, Usio, 2000, Usio and Townsend, 2002). Decapods are also consumers of other, smaller macroinvertebrate shredders (Haddaway et al., 2012, Rosewarne et al., 2013) which potentially could affect total processing of leaf litter. Invasive alien species of decapod are widespread in freshwaters globally where they often extirpate native decapods (Dunn et al., 2009, Lodge et al., 2012, Twardochleb et al., 2013).

Invasive species often differ from analogous natives in physiological and behavioural traits (e.g. (129)Dick et al. 2014), and in their trophic position (Tran et al., 2015). Many studies on leaf litter processing by invasive alien decapods have looked only at the invasive species without comparing processing by invasives to the analogue native, which may be extinct (Rudnick and Resh, 2005, Schuster, 2010, Moore et al., 2012). Exceptions when native analogues are compared to invasive species, responses appear to vary between invasive species (Dunoyer et al. 2014). These studies predominately focused on decomposition rates and less on the derived products. Significant production of fine particulate organic matter (FPOM) can result from the passage of leaf litter through the crayfish gut (Usio et al., 2001, Montemarano et al., 2007, Carvalho et al., 2016). Decapod crustaceans excrete ammonia and other nutrients (Kristiansen and Hessen, 1992) and fragmentation of leaf litter increases organic matter surface area for microbial activity. Released nutrients are then made available to primary trophic levels such as microbes and algae (Dyson et al., 2007, Kominoski et al., 2014, Woodward et al., 2012). This further decomposition of excreted FPOM potentially influences regulating ecosystem services (such as nutrient cycling), but has rarely been taken into account for aquatic invasive alien species. Different species produce different amounts of biologically active excreted nutrients, hence changes in species composition are expected to affect nutrient cycling (McIntyre et al., 2007), especially in biological invasions (Capps and Flecker, 2013). However, the extent to which differential (native versus alien) decapod shredding alters concentrations of nutrients in the water column, either directly or indirectly remains to be studied.

In the British Isles and mainland Europe west of the Alps, the indigenous White Clawed Crayfish, *Austropotamobius pallipes* shows significant declines with the advance of the American Signal Crayfish, *Pacifastacus leniusculus*, which are followed by invasions of the Chinese Mitten Crabs (*Eriocheir sinensis)* (Rogers and Watson, 2010, Almeida et al., 2014). If these invasive decapods differ in their leaf litter shredding function, this could have profound impacts on whole river basin resource processing rates and wider food webs. *A. pallipes* are often infected with the microsporidian parasite, *Thelohania contejeani*, which causes Porcelain Disease that manifests in lethargic behaviour and reduced feeding rates (Imhoff et al., 2009, Haddaway et al., 2012). This parasite can infect *P. leniusculus*, but disease does not develop (Imhoff et al., 2012). Therefore the impact of *T. contejeani* infection on resource processing by native White Clawed Crayfish must be considered as a confounding factor potentially driving differences in detritivory performances.

This study investigated the impact of these native versus invasive alien freshwater decapod crustaceans on the processing of allochthonous resources (abscised leaf litter) and the consequences of this on lower trophic levels (biofilm). The study tested the following hypotheses:

(H_i) the two invasive alien species consume and breakdown more leaf litter than native crayfish or decapod-free controls due to their higher metabolic rates compared to the native crayfish (Rosewarne et al., 2014).

(H_{ii}) Production of secondary products (smaller leaf fragments [coarse particulate organic matter – CPOM], fine particulate organic matter [FPOM] and dissolved nutrients) would be higher in invasive alien species treatment due to their increased consumption, thus excretion rate.

(H_{iii}) Enhanced production of secondary products in the invasive decapod treatments would have a positive impact on the biomass of biofilm, via dissolved nutrients fuelling metabolism and growth.

(H_{iv}) Leaf breakdown and nutrient release were expected to be lower for infected than uninfected *A. pallipes* because parasitism by *T. contejeani* leads to reduced activity and feeding rates (Haddaway et al 2012).

2.2 Methods

2.2.1 Materials

Alder (*Alnus glutinosus*) leaves collected from around the University of Leeds campus were oven dried at 50^{o}C and stored in paper bags prior to experimentation. Alder was chosen as it is a dominant native riparian species in Europe, and is commonly used in studies on decomposition rates (Abelho, 2001). Leaves were subsequently combined into pre-weighed packs (3.0-3.5 g), placed in a labelled mesh bag, and conditioned (*sensu* Gessner et al 1999) in water from a nearby stream (Meanwood Beck, Leeds: 53.820937 N, 1.5604979 W) for two weeks for microbial communities to colonise.

Chinese Mitten Crabs were obtained from the River Thames at Chiswick, London (51.488489 N, 0.24471175 E). American Signal Crayfish were collected from Fenay Beck, Huddersfield (53.641660 N, 1.7310895 W). White Clawed Crayfish were collected from Clapham Beck, North Yorkshire (54.117165 N, 2.3921497 W), Wyke Beck, West Yorkshire (53.827819 N, 1.4893696 W) and Adel Beck, West Yorkshire (53.855078 N, 1.5743397W). All animals were captured by hand, held in the laboratory for a minimum of two weeks prior to experimentation, and fed Crab Cuisine® pellets *ad libitum*. Animals were starved for 24 hours prior to initiation of the experiment.

Biofilm was obtained from unglazed stone tiles (22 X 22 X 10 mm, with a reactive surface area of 1364 mm^2) incubated outdoors for 3 months (July-October 2014) in a water tank seeded from a nearby lake (Wothersome Lake –53.874944 N, 1.3913008 W) and pond (Kirkstall Valley Nature Reserve, Leeds, 53.811316 N, longitude: 1.6032428W). The tiles were then brought into the laboratory cold room (see below) for two weeks to acclimatise. A subset of 25 tiles was sampled to measure basal biofilm at the start of the experiment. A single biofilm-colonized tile was added to the lower chamber of each microcosm, to prevent crayfish disturbing or consuming this resource (Gherardi and Lazzara, 2006, Burns and Walker, 2000). Another clean tile was placed next to the pre-colonized tile to measure growth of new biofilm during the experiment. Microcosms were placed on top shelves of the cold room to avoid shade and ensure maximum exposure to light in the room (photosynthetically active radiation= 1.05 ± 0.85 μmol s^{-1} m^{-2}, measured with a PAR Quantum Radiometric probe, Skye Instruments), at the lower end of the range in natural streams (Hauer and Hill, 2006).

2.2.2 Experimental design

Microcosms were set up in the lab to compare the effects of native and invasive alien decapod species on leaf litter processing in a controlled environment. Microcosms consisted of 30 X 20 X 15cm (4 litres) plastic tanks containing aerated dechlorinated tap water. Microcosm chambers consisted of an upper section containing leaf litter, a single decapod crustacean and a PVC pipe for shelter; and a lower chamber separated by 1 mm aperture nylon mesh (Fig 2.1). The mesh allowed FPOM particulates <1 mm to pass through whilst retaining larger leaf litter fragments in the upper chamber for further consumption. The mesh also served to isolate the lower chamber and accumulated FPOM and biofilm tile from the decapod. Microcosms were incubated at 14^{o}C on a 16:8 hour light:dark cycle.

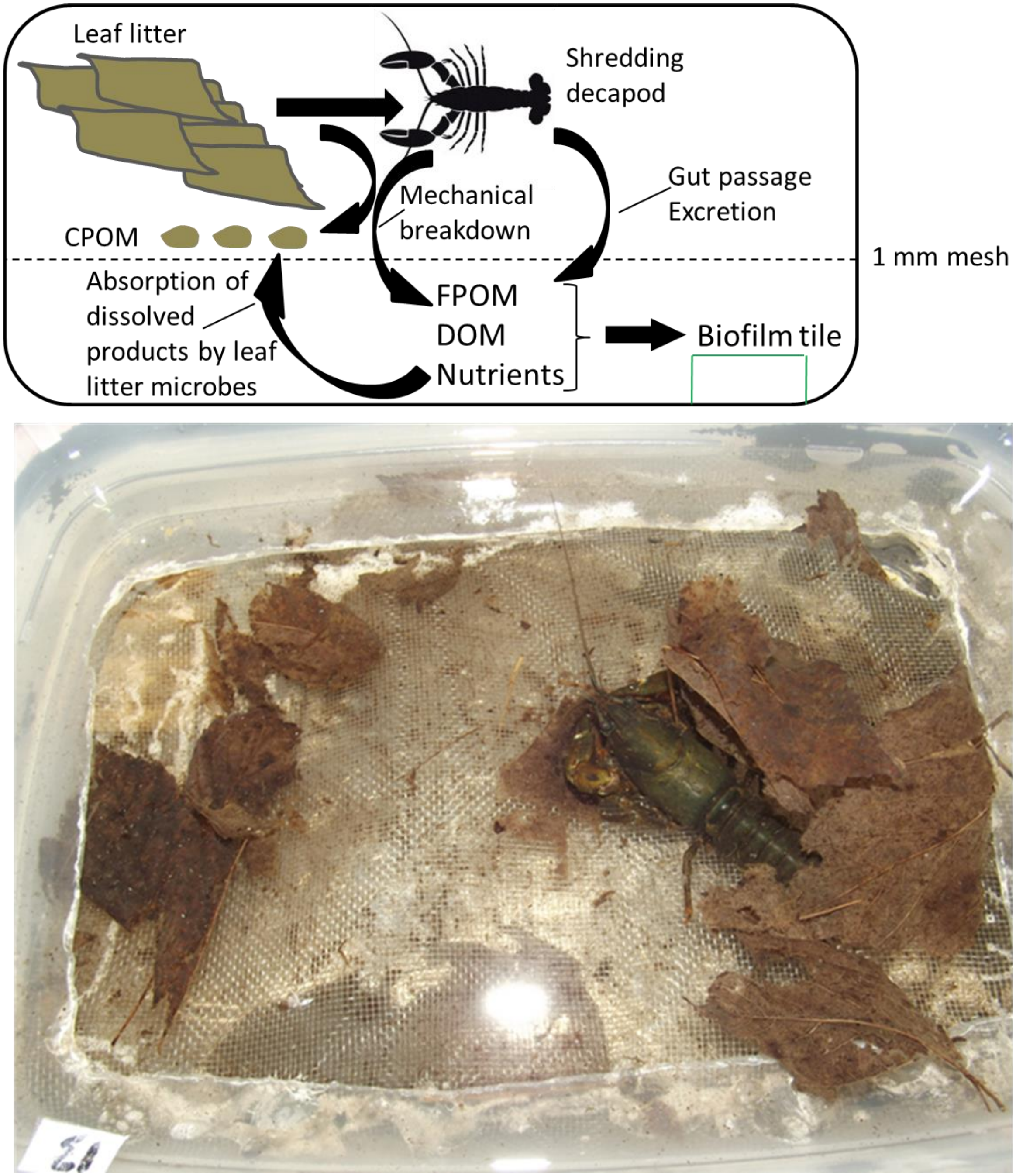

Figure 2.1. Microcosms used in the experiment.

Five treatments were established : (1) White Clawed Crayfish, *A. pallipes* (native crayfish); (2) *A. pallipes* individuals visibly showing disease from infection by *T. contejeani* (native parasitized crayfish) (Imhoff et al., 2009); (3) American Signal Crayfish, *P. leniusculus* (alien crayfish); (4) Chinese Mitten Crab, *Eriocheir sinensis* (alien crab); and (5) a control with no decapods, to measure the effects of microbial conditioning and (to a lesser extent, *sensu* Gessner et al 1999) leaching. Sub-adult decapods (mass range 1.49-19.8 g) were added to microcosms following a randomised design. This age class forms a significant proportion of populations of all these species (personal observation). All treatments consisted of 24 replicates (with equal representation of males and females), with the exception of Chinese Mitten Crabs (13 replicates; 6 males and 7 females) and diseased White Clawed Crayfish (1 male, 10 females). The experiment ran for 14 days. On day 7, water was changed through siphoning to ensure welfare of decapods by removing toxic excreta to maintain optimal performance. To prevent the introduction of confounding dilution effects, equal volumes of water were siphoned and replaced in each chamber.

After 14 days, measurements were taken of: decapod mass, remaining leaf litter mass, smaller CPOM fragments mass, FPOM mass, nutrient concentrations (organic and inorganic carbon, ammonia, nitrate, soluble reactive phosphorous) and biofilm.

To measure decomposition rates, CPOM and FPOM production, the contents of the upper chamber were rinsed in microcosm water by gently moving the mesh to ensure that all FPOM dropped to lower chamber. Remaining leaf litter was placed in labelled paper bags. Smaller leaf fragments (CPOM 1-10 mm) in the top layer of the microcosm were also collected using tweezers and placed in paper envelopes. Leaf litter and CPOM were dried at 50^{o}C, weighed and ashed at 500^{o}C to estimate ash-free dry biomass. Decomposition rate (AFDM per day) was calculated by subtracting final AFDM from the starting AFDM of leaf litter, following (Benfield, 2006). Fine particulate organic matter (FPOM) was sampled from a 50ml aliquot of the homogenized microcosm water, filtered on a pre-ashed 0.7 µm GF/F filter disc, and ash-free dry biomass estimated following Ramchunder et al. (2011).

Water samples were taken from the water column, filtered through 0.45 µm nylon filters and tested for dissolved nutrients (NH_3-N, NO_3-N, PO_4-P) measured using a Skaler SAN ++ continuous flow auto-analyser; and dissolved organic carbon (DOC) using a Analytik Jena Multi NC2100 combustion analyser. To compare decapod nutrient excretion rates in the absence of microbial biofilms on leaf litter and tiles that would absorb nutrients, a separate incubation was set up for the treatments ($n = 10$) as above (except for parasitised crayfish). Decapods unfed for 24 hours were incubated in dechlorinated tap water for 24 hours with a decapod-free control, after which water samples were taken and processed for dissolved nutrients (NH_3-N, NO_3-N, PO_4-P) as described above, following modified protocols of Vanni et al. (2002).

To measure response of biofilm, tiles were removed and the biofilm sampled from each separately using a firm nylon brush rinsed with deionised water, making up to 50ml solution. A 5ml sub-sample of the homogenised slurry was then filtered on to pre-ashed GF/F filter discs (0.7 µm) and chlorophyll *a* extracted in dark conditions then measured using a portable spectrophotometer (Hach DR/2010) following Steinman et al. (1996). The remainder of the slurry was filtered as for FPOM, dried in an oven at 105^{o}C and ash free dry biomass (AFDM) estimated from loss on ignition at 500^{o}C (Steinman et al., 1996).

2.2.3 Data analysis

All data were analysed using R (R v.3.1.0.; R Development Core Team, 2014). Generalised Linear Models (formula: glm) were used to compare the response variables (leaf litter decomposition rate, CPOM production, FPOM production, dissolved nutrients, biofilm productivity) against the different treatments. Post-hoc Tukey tests were used to test for differences between treatments. For those response variables that showed significant relationships, effect size was calculated using Cohen's d (Cohen, 1992). Effect sizes of 0-0.2 are interpreted to be negligible, 0.21-0.79 moderate and > 0.8 strong in either the negative or positive direction (Cohen, 1992).

To factor in the body mass of decapods for decomposition rate, detritivory performance (also termed shredder efficiency, *sensu* Piscart et al 2011 and MacNeil et al 2011: ash-free dry mass of leaf litter lost per day per mass of animal) was calculated by subtracting the mean decomposition rate in decapod free controls from the actual decomposition rate for each replicate, and then dividing that by the starting wet mass of the animal:

$$Detritivory\ performance = \frac{\lambda t - \lambda c}{mt}$$

where λt is the decomposition rate (ΔAFDM g/day) for replicate *t*, and λc is the mean decomposition rate for all control (leaching and microbial decay) treatments, and *mt* is the mass of the animal in replicate *t*. Efficiencies of production of FPOM and DOC were calculated using same equation except decomposition rate replacing these variables.

2.3 Results

2.3.1 Leaf litter decomposition

Leaf litter decomposition rate differed significantly between species (Table 2.1), being greater for the invasive alien *E. sinensis* and *P. leniusculus* compared to the native *A. pallipes* and the control (Fig. 2.2a). Effect sizes were large for all decapod treatments, but markedly higher for invasive alien compared to native species (Fig. 2.3). There was a significant species-mass interaction (GLM p = 0.001, Table 1), with *P. leniusculus* decomposition rate more strongly correlated with mass (GLM p > 0.001, linear regression: R^2=0.42, coefficient=0.2775, Fig. 2.4) than *A. pallipes* (including parasitized individuals, p = 0.01, R^2=0.15, coefficient=0.0794, Fig. 4). Detritivory performance (g AFDM loss per day per mass of decapod) differed significantly between species (Table 2.1) and was significantly higher for the invasive alien decapods, but did not differ between diseased and undiseased crayfish or between the two invasive alien decapods (Supp. Tab. 2.1, Fig. 2.2b). Change in mass of decapods during the experiment did not vary significantly between the species treatments (Table 2.1).

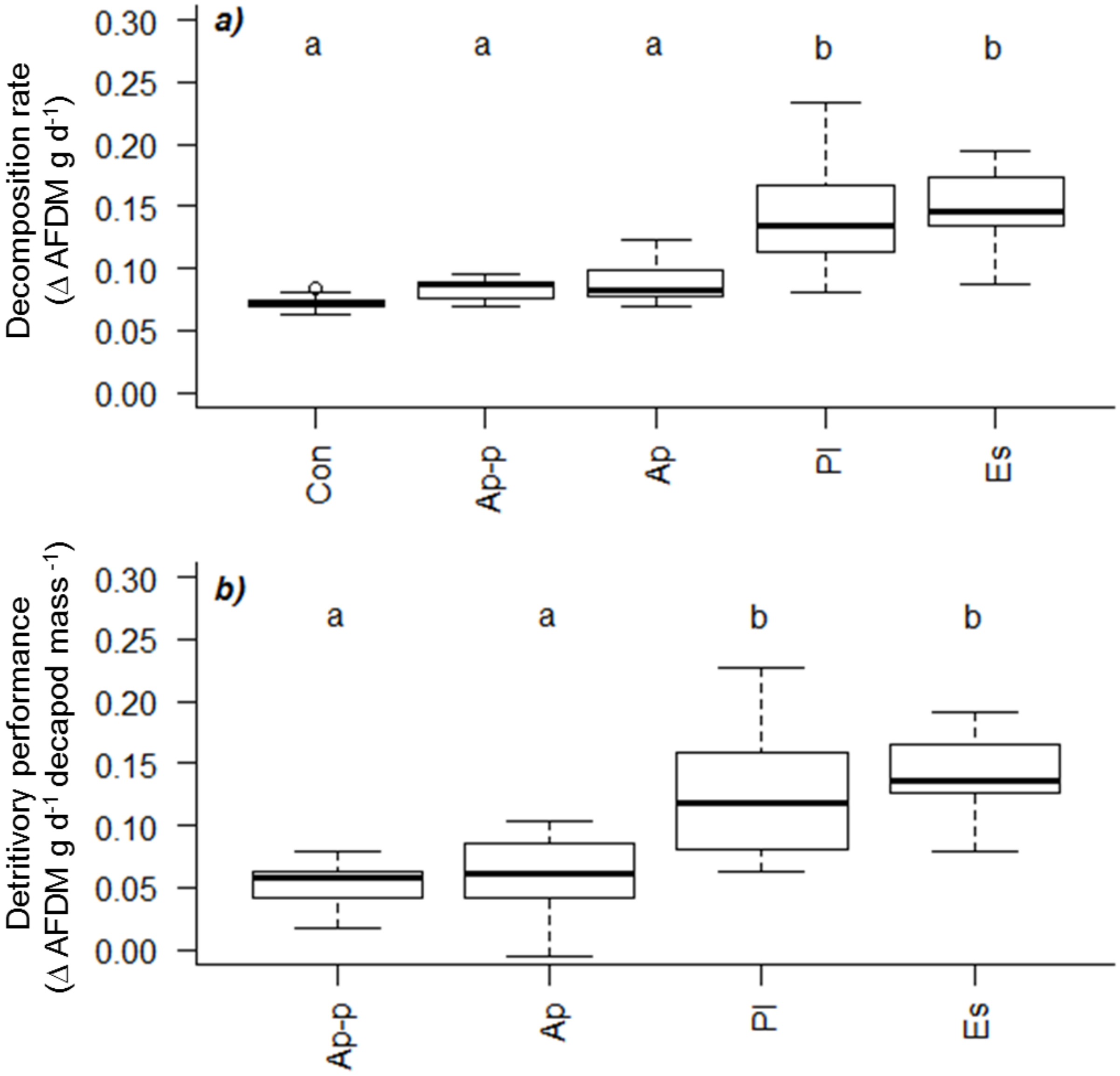

Figure 2.2. Processing of leaf litter quantified from measurements of: a) decomposition rates (g loss of AFDM per day), b) detritivory performance of each treatment, (g loss of AFDM per day per g of animal). Abbreviations on x-axes: Con –control; Ap-p – native crayfish (*A. pallipes*) showing no signs of disease; Pl – alien invasive crayfish (*Pacifastacus leniusculus*); Es – alien invasive crab (*Eriocheir sinensis*). Letters above boxplots indicate groupings based on *post-hoc* tests (see Supplemental Table 1).

Table 2.1. Generalized linear models with experimental treatment as the factor

Hypothesis	**Response variable**	**Effects model**	**df**	**Residual deviance**	**Pr(>Chi)**	**AIC**
1)Breakdown of leaf litter	Decomposition rate	Species	4	0.052	<0.001	-438
		Mass	1	0.043	**<0.001**	-292
		Species*mass	3	0.007	**0.001**	-339
	% change in mass	Species	3	677.520	0.476	613
		Mass	1	266.84	0.320	611
		Species*mass	3	787.34	0.403	616
	Detritivory performance	Species	3	0.102	**< 0.001**	-
2) Secondary products	CPOM (10-1mm size) production	Species	4	0.235	**< 0.001**	-343
		Mass	1	0.052	**<0.001**	-232
		Species*mass	3	0.013	0.053	-247
	FPOM production	Species	4	7.692	**<0.001**	180
		Mass	1	18.682	**<0.001**	167
		Species*mass	3	3.411	**0.026**	140
	FPOM production efficiency	Species	3	12.563	**< 0.001**	-
	DOC production	Species	4	58.56	**0.022**	412
		Mass	1	41.41	**<0.001**	304
		Species*mass	3	13.86	0.295	309
	DOC production efficiency	Species	3	0.850	**0.002**	-
	Ammonia (NH_4-N)	Species	4	4.501	0.993	-274
		Mass	1	0.033	**<0.001**	-208
		Species*mass	3	0.035	**0.003**	-216
	Nitrate (NO_3-N)	Species	4	23.86	0.346	150
		Mass	1	0.010	0.848	121
		Species*mass	3	3.443	**0.005**	118
	Phosphate (PO_4-P)	Species	4	3.061	**< 0.001**	-129
		Mass	1	0.164	**0.001**	-88
		Species*mass	3	0.107	0.055	-92
3) Biofilm response	Biomass accrual	Species	4	0.045	0.269	-
	Primary productivity	Species	4	13.94	0.828	-
	Biomass of establish tiles	Species	4	0.003	0.085	-
	Primary productivity of establish tiles	Species	4	49.08	0.577	-

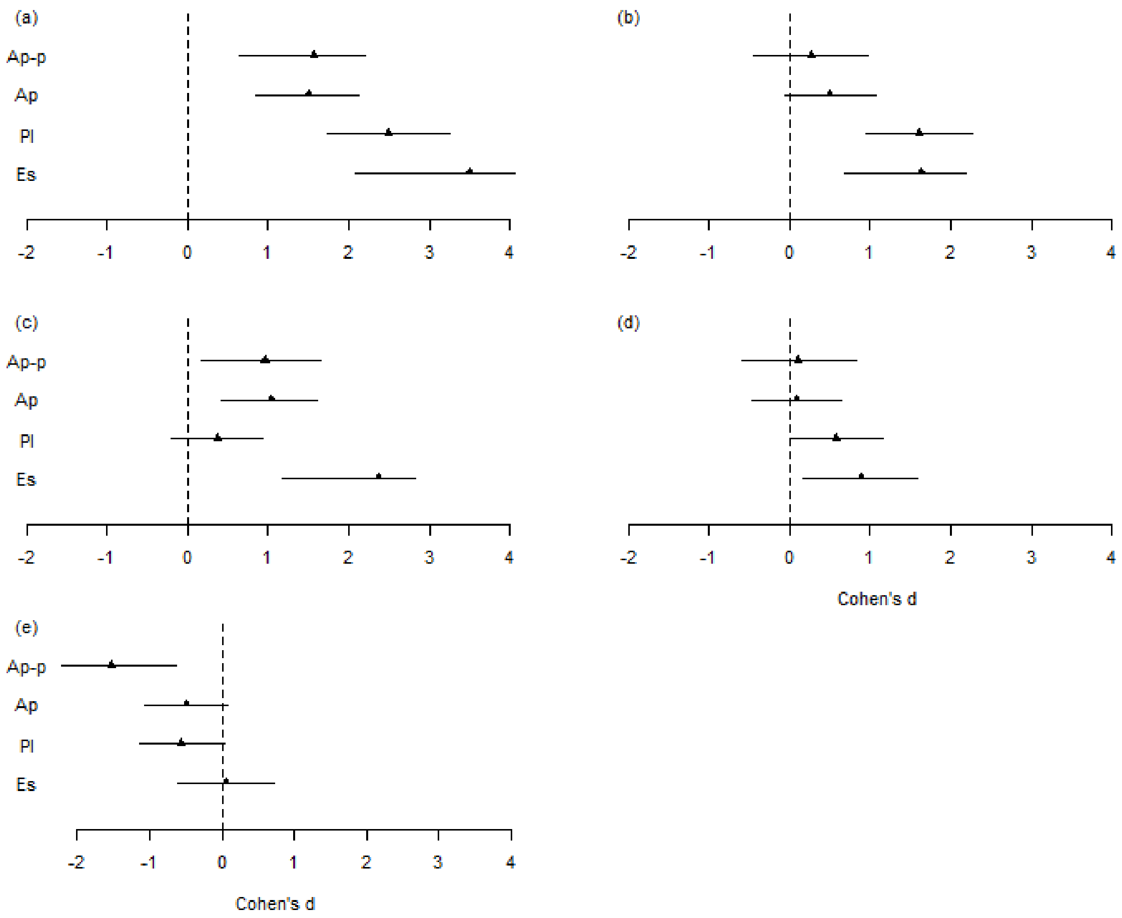

Figure 2.3. Effect sizes (Cohen's *d*) for variables showing significant relationships with the experimental treatments: a) leaf litter decomposition rate; b) production of CPOM; c) production of FPOM; d) Dissolved organic carbon; e) Phosphate. Abbreviations on the y-axis are the same as those used in Fig.2.

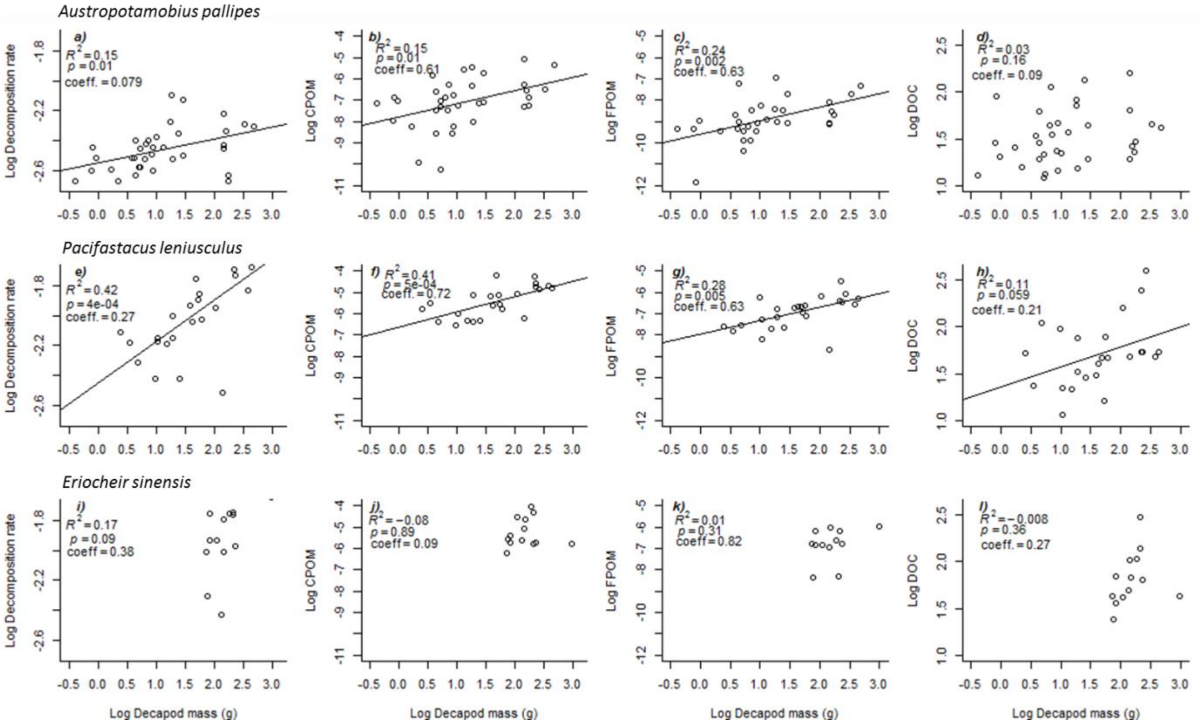

Figure 2.4. Allometric scaling of leaf litter processing across freshwater decapod species: Showing decomposition rates (a,e,i), CPOM production (b,f,j), FPOM production (c,g,k) and DOC production (d,h,l) for *Austropotamobius pallipes* (a-d) (including individuals showing disease from *Thelohania contejeani* as these did not differ in detritivory performance to undiseased individuals), *Pacifastacus leniusculus* (e-h) and *Eriocheir sinensis* (i-l).

2.3.2 Secondary products from detritivory

Production of CPOM fragments was significantly affected by decapod species and by decapod mass but there was no significant mass-species interaction (Table 2.1, Fig. 2.5a). Effect sizes were high for both invasive alien species (Fig. 2.3b). FPOM production was significantly affected by species, by mass and there was a significant species-mass interaction (Fig. 2.5b, Table 2.1). Efficiency of FPOM production (AFDM g/day/mass of decapod) was significantly higher for both invasive alien species (Fig. 2.5c, Table 2.1).

Dissolved organic carbon concentration differed significantly between the species treatments, (Figs. 2.3d & 2.5d), and was affected by mass (Table 2.1). DOC production efficiency (daily production of DOC per mass of animal) differed significantly between species, being higher in *A. pallipes* compared to both invasive alien decapods (Supp. Tab. 2.1).

Dissolved nutrients (ammonia, nitrate) did not differ between treatments, though ammonia did significantly, positively correlate with decapod mass (Table 2.1). Phosphate differed between species treatments (Table 2.1, Fig. 2.6), being significantly lower in parasitized *A. pallipes* (effect size, *d*, = -1.52), moderate in unparasitised *A. pallipes* (*d* = -0.49) and *P. leniusculus* (*d* = -0.55) and negligible in *E. sinensis* (Fig. 2.3f). Phosphate had a significant, positive relationship with decapod mass (Table 2.1). These negligible differences in nutrient concentration following detritivory contrast to those from excretion rates between the decapod species (Table 2.2, Fig. 2.6). Both *A. pallipes* and *E. sinensis* had a higher excretion rate of ammonia than *P. leniusculus*, which showed ammonia levels similar to decapod-free controls (Fig 2.6a). Nitrates were elevated in all decapod species compared to controls (Fig 2.6b). Phosphate excretion was significantly lower for *E. sinensis* compared to the other decapod species (Fig 2.6c). The only nutrient excreted that had a significant, positive relationship with decapod mass was ammonia (R^2=0.21, Table 2.2).

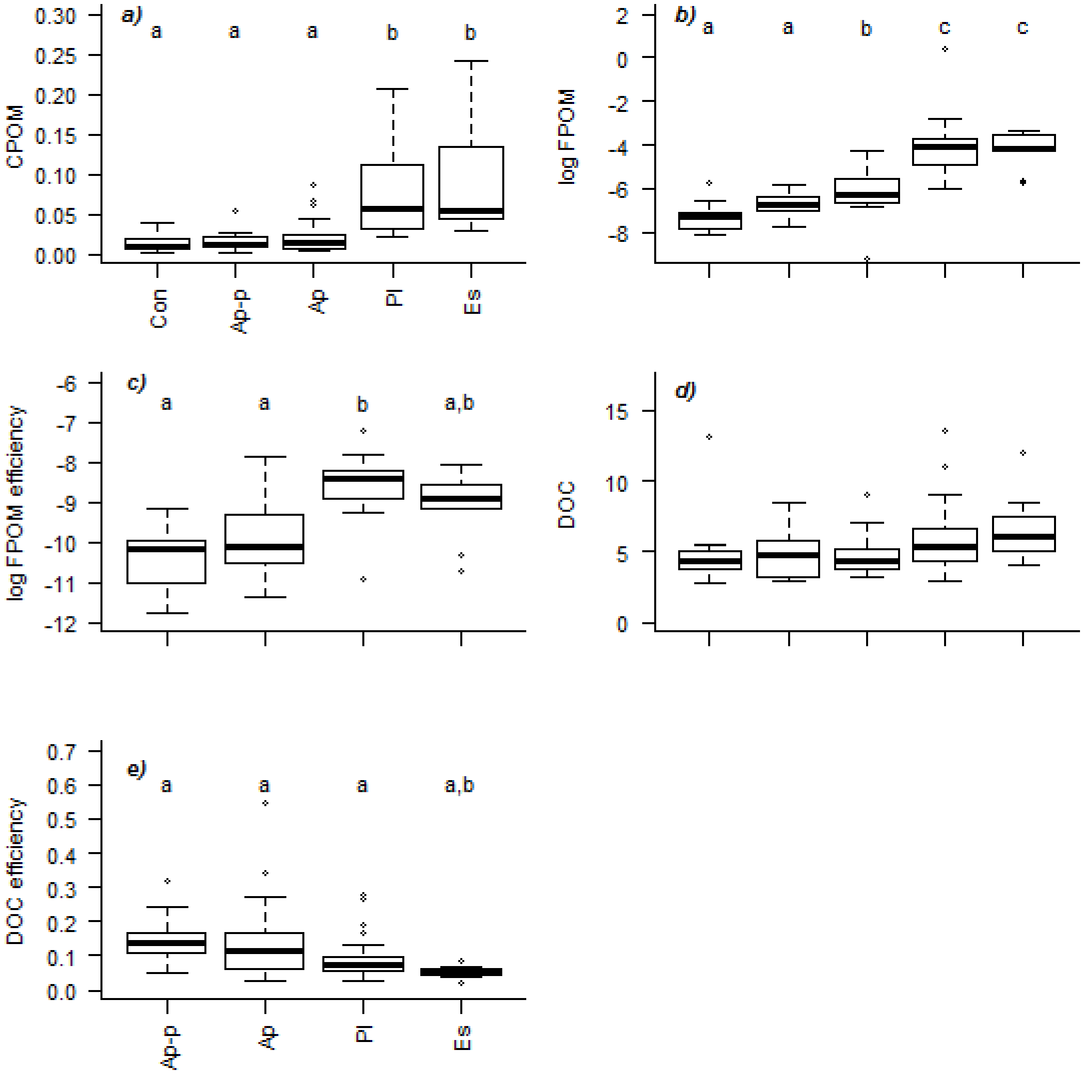

Figure 2.5. Products derived from detritivory. a) production of smaller fragments (CPOM 10-1mm) (g AFDM per day), b) $\log_{10}$ FPOM (1mm-0.7 µm) production per day (g AFDM per day); c) $\log_{10}$ FPOM production efficiency (daily production of FPOM divided by mass of decapod - g AFDM per day per decapod mass); d) dissolved organic carbon (mg/L); e) efficiency of DOC production (mg/L/mass of animal). Abbreviations of x-axis and letters above box-plots as per Fig. 2.2. Letters above boxplots indicate groupings based on *post-hoc* tests (see Supplemental Table 2.1).

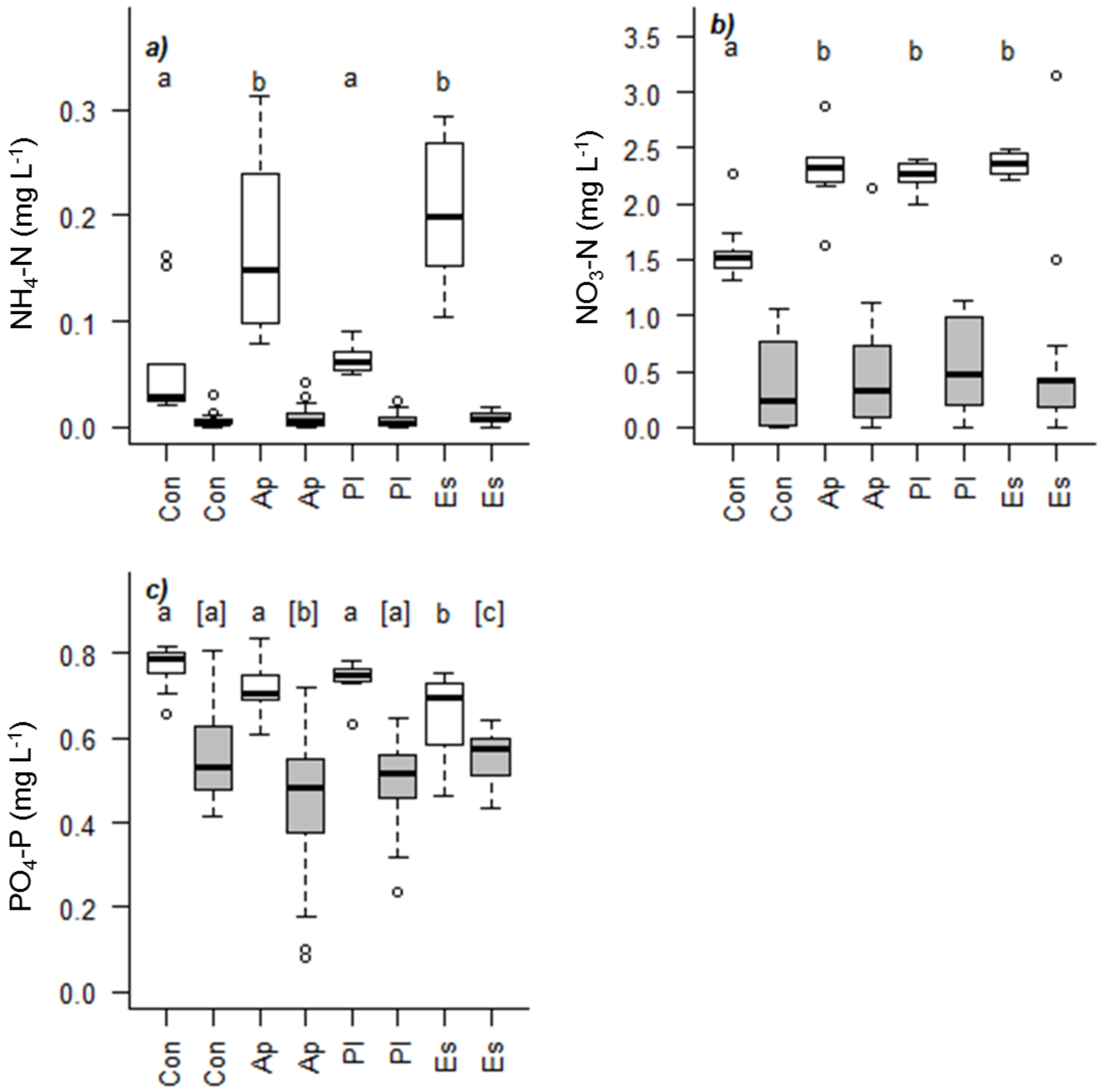

Figure 2.6. Nutrient production rates for incubations without leaf litter and biofilms (white bars) and for the main experiment in the presence of leaf litter and biofilms (grey bars). Letters above boxplots indicate groupings based on *post-hoc* tests, with those in parentheses distinguishing the detritivory experiment and those without the incubation for excreta (see Supplemental Table 2.1). Abbreviations for x-axis as for Fig 2.2.

Table 2.2. Generalised linear models for nutrient excretion for native and invasive alien decapod treatments (*post-hoc* test results in Supplemental Table 2.2)

Response variable	**Effects model**	**df**	**Residual deviance**	**Pr(>Chi)**	**AIC**
Ammonia (NH_3-N)	Species	3	1.163	**>0.001**	-85
	Mass	1	0.044	**0.006**	-53
	Species*mass	2	0.031	0.512	-58
Nitrate (NO_3-N)	Species	3	3.512	**>0.001**	4
	Mass	1	0.127	0.078	4
	Species*mass	2	0.102	0.311	0
Soluble Reactive Phosphorous (PO_4-P)	Species	3	0.082	**0.011**	**-77**
	Mass	1	0.003	0.464	-50
	Species*mass	2	0.002	0.801	-47

2.3.3 Biofilm response

Primary productivity and biomass on both established and accrued biofilms did not differ between treatments (Table 2.1), though there was a trend for higher biomass of established biofilms in treatments with healthy native crayfish and alien decapods in relation to parasitized crayfish and controls (Suppl. Fig. 2.2). All tiles used in the experiment showed significantly (GLM $p > 0.001$) increased (Cohen's $d > 1$) growth of biofilm biomass (AFDM) but not chlorophyll *a* in relation to the tiles not exposed to conditioned leaf litter, regardless of treatment (Suppl. Fig. 2.2). Biofilm mass and chlorophyll *a* showed weakly positive but significant correlation with decomposition rate, nitrate and phosphate (summarised in Suppl. Table 2.3).

2.4 Discussion

This study demonstrates differences in shredding by native and invasive decapods which led to changes in the availability of resources. The two invasive alien species showed higher leaf litter decomposition rates in relation to native crayfish and controls, with increased secondary products: CPOM, FPOM and DOC. However, biofilm growth was not affected by this differential resource availability.

Breakdown rates of leaf litter were higher for the invasive alien decapods *P. leniusculus* and *E. sinensis*, being almost double the rates for native crayfish of equivalent age classes. This supports the hypothesis of higher rates of breakdown by the invasive alien species. Although parasitism can lead to changes in the host resource consumption rates, parasitism had not effect on the breakdown of litter by *A. pallipes*. Efficiency of breakdown of leaf litter, even after factoring in the effect of body mass, was also greater in invasive alien decapods compared to the native, suggesting a trait-based cause for enhanced decomposition in addition to body size. These observations are consistent with previous comparisons of invasive to native decapods (Bondar et al., 2005, Rudnick and Resh, 2005, Moore et al., 2012, Dunoyer et al., 2014). Given that *P. leniusculus* and *E.* sinensis reach higher mass than the native *A. pallipes*, the difference in leaf litter decomposition rates could be greater in the field.

The impact of these invasive alien decapods in the field will also be influenced by the presence of alternative food sources, and their predatory effect on other smaller shredding species. This predatory effect has been shown to be higher for the *P. leniusculus* and *E. sinensis* than for the native *A. pallipes* (Haddaway et al., 2012, Rosewarne et al., 2016). In one lab experiment, Dunoyer et al. (2014) reported higher leaf litter decomposition rates when both *P. leniusculus* and amphipods (*Gammarus pulex*) were present, than when only the crayfish was present. In contrast, red swamp crayfish *Procambarus clarkii* depleted shredding invertebrates at the expense of enhanced decomposition (Dunoyer et al., 2014). It is thus expected that the current experiment underestimates the impact of American Signal Crayfish on leaf litter decomposition rates in the field.

Smaller fragments of CPOM (10-1mm) were produced as a result of shredding, with more CPOM produced in the invasive alien decapod treatments. This organic matter is likely to be consumed by other shredders (such as amphipods, MacNeil et al., 2011, Piscart et al., 2011). Production of FPOM and DOC was also higher in the invasive decapod treatments, supporting the hypothesis that higher leaf breakdown by the invaders would lead to greater production of secondary products. This is likely a result of consumption and processing of leaf material through the decapod gut, as opposed to mechanical breakdown (Montemarano et al., 2007) with nutrients absorbed by the gut of the decapod, and finer material excreted to form FPOM and DOC, as well as any unabsorbed nutrients.

P. leniusculus showed high decomposition rates but contrastingly lower effects on FPOM production suggesting a greater rate of assimilation of leaf litter than other species, but with higher impacts on DOC production, perhaps suggesting even finer excreted material. While ammonia concentration did not vary between species in the decomposition experiments, its excretion did differ, with the low excretion by *P. leniusculus* indicating this species could act as a nitrogen sink (Vanni, 2002). The negligible ammonia concentrations in the presence of leaf litter are likely a result of absorption by microbial biofilms growing on both tiles and leaf litter, as well as nitrification. *E. sinensis* showed reduced phosphate excretion, suggesting a greater phosphorous ratio in the body mass, indicating this species could be a phosphorous sink.

Abscised alder leaves typically have higher nitrogen ratios compared to most other leaf species (Hladyz et al., 2009), but release of this nitrogen following processing by decapods was not detected in the water column. It is possible that the nitrogen in the leaf litter was assimilated by decapods, particularly *P. leniusculus*, for nitrogen-rich chitin exoskeletons, or that growing biofilms consumed the nitrogen. However, neither of these relationships were observed based on decapod mass change nor biofilm productivity, respectively. Crayfish of other genera (*Oronectes*) excrete products with a high N:P ratio, and likely increase nitrogen (ammonia, nitrate and total nitrogen) concentrations in the water column (Evans-White and Lamberti, 2005). Differences in excretal products could also have been obscured by absorption of nutrients by microbes on the leaf litter (Mehring et al., 2015).

Consumer specific differences in production of secondary products did not explain variation in either biofilm biomass or primary productivity. This is in contrast to previous studies which found that biofilms exposed to nitrogen-rich excretal products of crayfish had higher primary productivity Evans-White and Lamberti (2005). A reduced productivity of biofilms despite the presence of increased DOC and nutrients could be explained by less light penetrating the water (Fig. 2.7). A similar relationship has been shown in boreal lakes, where terrestrially-derived dissolved carbon can suppress productivity, due to increased DOC reducing autochthonous production (Karlsson et al., 2015). Although no response of biofilm to FPOM and DOC produced by the decapods was found, other trophic levels may be affected. Absorber guilds, such as phytoplankton or fungal hyphomycetes might consume the products derived from enhanced leaf litter decomposition by decapods (Mehring et al., 2015). Collector-gatherers consuming FPOM might also benefit, especially filter-feeders such as bivalves (Gergs and Rothhaupt, 2008), certain amphipods (Navel et al., 2011) and chironomid larvae (Rosi-Marshall, 2004).

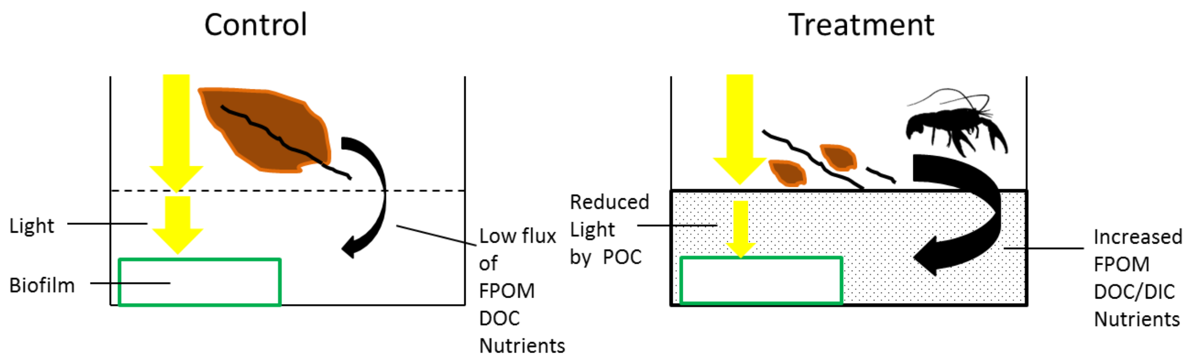

Figure 2.7. Hypothesised interaction between light, detritivory and biofilm productivity. The net result is similar productivity of biofilm in both controls and treatments but because of differing causes: high light and low nutrients for the control; reduced light but increase particulate organic matter and nutrients for the treatment.

This experiment has shown that both native and alien decapods enhance the breakdown of leaf litter into smaller fragments, including coarse and fine fragments, and dissolved carbon. This increase in secondary resources however did not affect the productivity of biofilms growing on tiles. Nevertheless, these results indicate that alien invasive decapods are not ecologically redundant when compared with native crayfish, and have the potential to significantly alter detrital processes, thus carbon cycling and food web productivity in freshwater ecosystems. This has the potential to alter river discontinuum patterns and processes for entire river basins.

Chapter 3. Invasive alien shredders clear up invasive alien plant litter

3.1 Introduction

Invasive alien species (IAS) threaten global biodiversity, ecosystems and economies, and multiple IAS may occur in an ecosystem across all trophic levels (Lowe et al., 2000, Simberloff et al., 2012a). Studies of the combined impacts of multiple invasive species on ecosystem functioning are limited, and are mostly restricted to between-species interactions with invasion success the primary outcome measured. Interactions between invasive alien species may be mutually facilitative (the "invasion meltdown" hypothesis, Simberloff and Van Holle, 1999). For example, the invasive alien American Signal Crayfish (*Pacifastacus leniusculus*) consumes the invasive alien Zebra Mussel (*Dreissena polymorpha*), but also consumes its competitors and both invaders increase in abundance (zu Ermgassen and Aldridge, 2011). Alternatively, IAS interactions may be antagonistic or neutral (Jackson, 2015). For example the decapod crustaceans *P. leniusculus* and *Eriocheir sinensis* have been found to consume resources complementarily to result in synergistic resource depletion (Rosewarne et al., 2016). Less well studied, are the effects of interactions between IAS at different trophic levels on ecosystem functioning.

In freshwater ecosystems, two prominent invasive alien species guilds are riparian plants and omnivorous animals (Gallardo et al., 2015). Invasive alien riparian plants impact freshwater ecosystem processes through reducing light levels (reducing primary productivity and changing thermal regimes) and through introducing allochthonous leaf litter that could be novel to detritivorous consumers either in quantity or quality (Hladyz et al., 2009, Hladyz et al., 2011). Equivocal decomposition rates of invasive plant litter have been observed in comparison to native leaf litter (Lecerf et al., 2007, Hladyz et al., 2009, Hladyz et al., 2011). Invasive alien omnivorous animals (including snails, amphipods, decapods, amphibians and fish) impact freshwater ecosystems through the direct consumption of detritus, primary producers, macroinvertebrates and small vertebrates, as well as trophic cascades and ecosystem engineering (Gallardo et al., 2015). Invasive alien omnivores can alter decomposition rates relative to native analogues, with invasive alien freshwater crayfish associated with greater decomposition rates (Dunoyer et al., 2014, James et al., 2015). Crucially, the combined effects of invasive riparian plants and aquatic detritivores has not been researched, undermining the ability to understand and manage the effects of invasions on freshwater food webs.

This study compared the processing of leaf litter from invasive alien riparian trees species by invasive alien freshwater decapods and contrasts these to equivalent native species. It was hypothesised that:

(i) decomposition rates would reflect the species-specific traits of the leaf litter, with slower rates for species with greater tannin and cellulose content, as observed by Hladys et al. (2009);

(ii) Invasive alien decapods would lead to higher processing rate of invasive alien leaf litter than native decapods due to greater consumption and metabolic rates (Haddaway et al., 2012, Rosewarne et al., 2014, James et al., 2015);

(iii) leaf species most consumed would facilitate decapods through increased growth.

3.2 Materials & Methods

3.2.1 Study system

This study considered a series of key freshwater fauna and flora of the British Isles, which have a long history of biological introductions and subsequent spread of invasions. Breakdown rates of leaf litter were compared for three riparian plants: the native Black Alder tree (*Alnus glutinosa*); the long-term alien Sycamore tree (*Acer pseudoplatanus*) and a more recent invasive alien shrub (*Rhododendron ponticum*). All these species are common in the riparian zones of lowland rivers and lakes throughout the British Isles. Shredding of these three species by three species of decapod was compared. The native shredding species was the White Clawed Crayfish (*Austropotamobius pallipes*), which is currently declining, with local extinction driven by competition and disease transmission from the advance of invasive crayfish (Dunn et al., 2009, Füreder, 2010, Rogers and Watson, 2010). The invasive alien shredding decapods were the American Signal Crayfish (*Pacifastacus leniusculus*) and the Chinese Mitten Crab (*Eriocheir sinensis*), both becoming dominant in rivers and lakes in the British Isles (Herborg et al., 2005, Rogers and Watson, 2010, Almeida et al., 2014, Holdich et al., 2014).

3.2.2 Experimental set up

Leaf litter was collected upon abscission around the University of Leeds. Leaves were dried at 50°C for 24 hours before being stored in cool, dry and dark places in paper bags. Fourteen days prior to the experiment, leaves were weighed to 1.5 g packs, placed in mesh bags and 'conditioned' with water from a nearby stream (Meanwood Beck, Leeds). *A. pallipes* were collected from Wyke Beck, Leeds. *P. leniusculus* were collected from Fenay Beck, Huddersfield. *E. sinensis* were collected from the River Thames, Chiswick. All decapods were kept in aged tap water for a minimum of 14 days prior to use in the experiment. Decapods were unfed 24 hours prior to use in the experiment.

Experiments were undertaken in microcosms consisting of 4 litre plastic tanks with aerated, aged tap water. A layer of 1 mm aperture nylon mesh separated the microcosm into two chambers. Decapods and leaf material were placed in the upper chamber. The mesh allowed fine particulate organic matter (FPOM < 1 mm; Montemarano et al., 2005) to fall through and become separated from the rest of the detritus (Fig. 1). Each microcosm contained one PVC pipe to act as a shelter for the decapods.

3.2.3 Experimental design and sample processing

A 4X3 experimental design was established with ten replicates per treatment. Leaf treatments were conditioned leaf litter of either *A. glutinosa*, *A. pseudoplatanus* or *R. ponticum*. Decapod treatments were of each decapod species (*A. pallipes* 12.87 ± 2.92 g, *P. leniusculus* 10.33 ± 2.98 g, *E. sinensis* 11.02 ± 4.28 g). Sub-adults were used because they are the most common age class encountered in the field. Controls with no decapods were also established. Leaf litter (1.5 g dry mass pre-conditioning) and an individual decapod were added to the upper chamber, then maintained at 14^{o}C with a 16:8 photoperiod for seven days.

At the end of the experiment, decapods were removed, weighed and remaining leaf litter removed and placed in paper bags. Smaller leaf fragments (coarse particulate organic matter [CPOM] – 10-1 mm sized fragments) from the main leaf that had not fallen through the 1 mm mesh were also collected with tweezers and retained. Microcosm water was then homogenized and a 50 ml aliquot removed to sample fine particulate organic matter (FPOM - >1mm). Water was then filtered through pre-weighed 0.7 µm GF/F filters. All leaf litter, CPOM and FPOM samples were dried at 50^{o}C, weighed, then ashed at 500^{o}C to estimate ash-free dry mass (AFDM).

3.2.4 Data analysis

Generalised linear models (formula: glm) were used to test the variation of decomposition rate (the change in AFDM of leaf litter per day from Benfield et al, 2006); CPOM, FPOM and change in decapod mass against leaf and decapod species treatment, with *post hoc* tests to differentiate between group differences. Because *A. pallipes* used in the experiment were slightly but significantly larger than the *P. leniusculus* and *E. sinensis* specimens (p = 0.01), decapod mass was included in the analyses as a co-variate.

3.3 Results

Decomposition rate and FPOM production differed both between leaf species and decapod species treatments (Table 3.1). CPOM production differed between decapods. There was a significant difference in the decomposition rates of the three leaf types: it was similar between the invasive alien *A. pseudoplatanus* and the native *A. glutinosa* and was lowest for *R. ponticum* (Fig 3.1a). Decomposition rate was also significantly affected by decapod species with higher decomposition rates for the invasive alien *P. leniusculus* and *E. sinensis* than for the native *A. pallipes* (Fig 3.1a). *Rhododendron* leaf litter decomposed significantly faster for *P. leniusculus* and *E. sinensis* treatments relative to controls and *A. pallipes*.

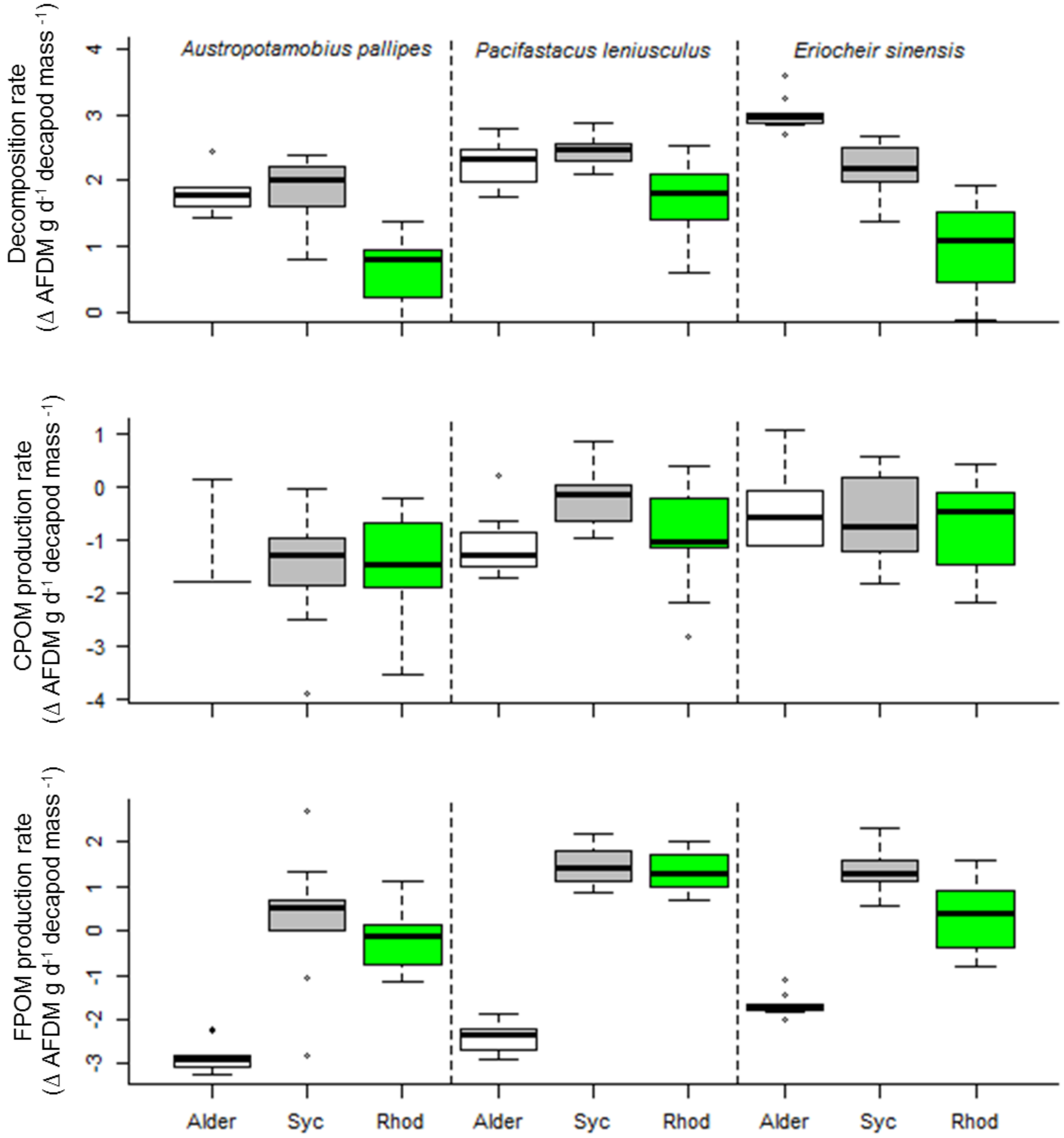

Figure 3.1. Leaf litter decomposition rates per decapod mass (a), CPOM production rate per decapod mass (b), and FPOM production rate per decapod mass (c) for Alder leaves (*Alnus glutinosa* - white bars), Sycamore leaves ("Syc" *Acer pseudoplatanus* - grey bars) and Rhododendron leaves ("Rhod." *Rhododendron ponticum* - green bars) in response to native crayfish *Austropotamobius pallipes*, invasive alien crayfish *Pacifastacus leniusculus* and invasive alien crabs *Eriocheir sinensis*.

Table 3.1. Generalised linear models summary statistics for decomposition rates (g decomposition/day), CPOM production (g/day) and FPOM production (g/day)

Response variable	Model	df	Residual deviance	Pr(>Chi)	AIC
Decomposition	Decapod Sp.	3	0.057	**<0.001**	-425
	Leaf Sp.	2	0.112	**<0.001**	-469
	Decapod X Leaf	6	0.006	0.123	-530
	Decapod X Mass	2	0.015	0.014	-306
	Leaf X Mass	2	0.000	0.878	-356
	Decapod X Leaf X Mass	4	0.011	**<0.001**	-394
CPOM	Decapod Sp.	3	0.000	**<0.001**	-942
	Leaf Sp.	2	0.000	0.328	-918
	Decapod X Leaf	6	0.000	0.348	-937
	Decapod X Mass	2	0.000	0.889	-682
	Leaf X Mass	2	0.000	0.948	-676
	Decapod X Leaf X Mass	2	0.000	0.399	-670
FPOM	Decapod Sp.	3	0.011	**<0.001**	-562
	Leaf Sp.	2	0.021	**<0.001**	-586
	Decapod X Leaf	6	0.007	**<0.001**	-624
	Decapod X Mass	2	0.011	**<0.001**	-407
	Leaf X Mass	2	0.000	0.588	-437
	Decapod X Leaf X Mass	4	0.001	0.536	-430
∆Decapod mass	Decapod Sp.	3	29.381	0.325	602
	Leaf Sp.	2	74.231	**<0.001**	595
	Decapod X Leaf	6	40.908	0.532	604

CPOM was significantly affected by decapod species only, with no interaction with leaf or decapod mass. Production of CPOM differed between decapod species treatment, but not between leaf species. Invasive alien decapods produced more CPOM than *A. pallipes* and controls (Fig. 3.1b). The mass of FPOM produced was significantly influenced by decapod species, leaf species and decapod mass, the former two factors interacted significantly to produce the model of best fit (Table 3.1). For all leaf species, significantly more FPOM was produced in treatments with invasive alien decapod species than in the native *A. pallipes* treatment and controls (Fig. 3.2c). *A. glutinosa* leaves produced significantly less FPOM compared to *A. pseudoplatanus* and *R. ponticum*, the latter two leaf species being similar to each other (Fig. 3.2c).

The change in decapod mass over the course of the experiment differed between leaf treatments, but did not differ between decapod species (Table 3.1). Decapods showed highest growth in the presence of *A. glutinosa* and *A. pseudoplatanus*, but showed negligible change in mass when provided with *R. ponticum*.

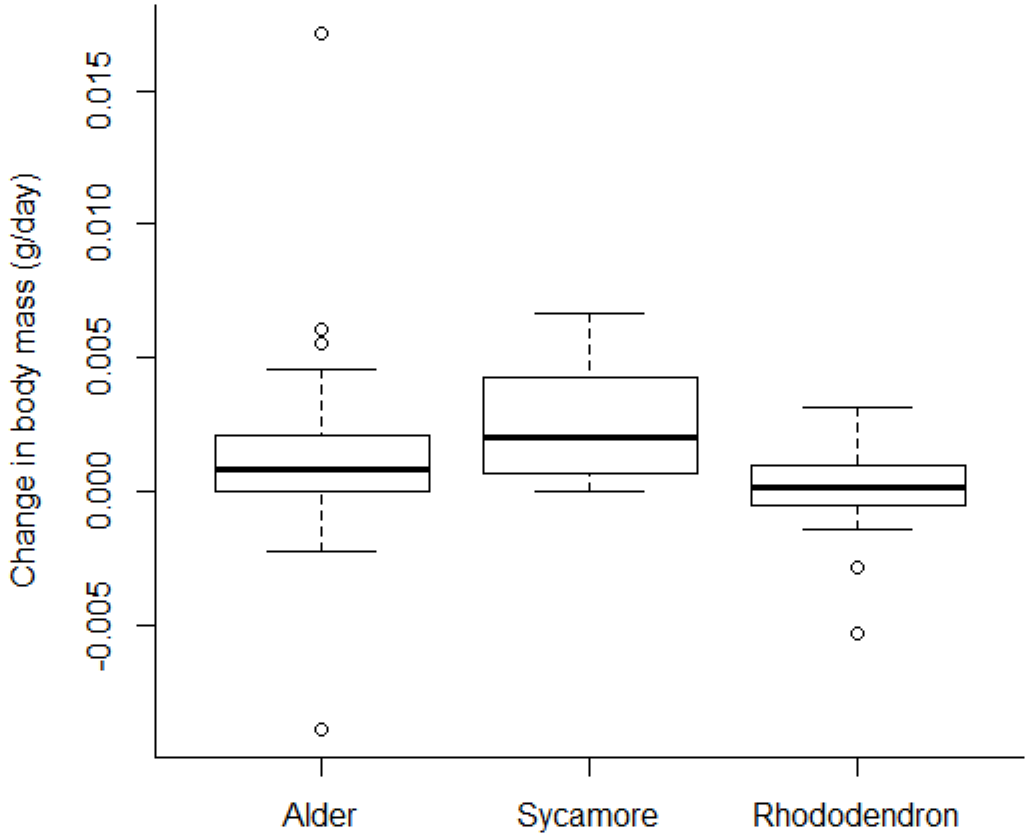

Figure 3.2. Change in mass of native and invasive alien decapods in relation to leaf species consumed.

3.4 Discussion

This is the first assessment of the interactions amongst invasive decapods and invasive alien leaf litter. The invasive alien decapods (*P. leniusculus* and *E. sinensis*) processed all species of leaf litter at a faster rate than the native analogue (*A. pallipes*), showing higher decomposition rates, and production of FPOM. This has implications for the removal and transformation of invasive alien litter to the remainder of the food web. These leaves would otherwise remain relatively unprocessed, potentially altering the structural heterogeneity of sediment beds, as well as starving detritivorous species. FPOM derived from leaf litter is a resource for collector-gathering invertebrates and accumulations of unprocessed leaf litter in headwaters has the potential to disrupt this energy flow across river basins (Vannote et al., 1980). Processing of invasive alien leaf litter by an invasive alien decapod would thus potentially neutralise one detrimental impact of invasive alien riparian plants incurred upon freshwater ecosystems.

Differences in leaf processing reflected leaf palatability as both *A. pseudoplatanus* and *R. ponticum* have greater cellulose and tannin content and they support lower fungal biomass and fewer macroinvertebrates compared with the native *A. glutinosa* (Hladyz et al., 2009, Hladyz et al., 2011). It is likely that that the greater FPOM mass from invasive alien treatments was the result of undigested leaf material passed through the gut of the decapod. This increased production of FPOM between leaf species is potentially caused by the reduced ability for crustaceans to digest leaf material in the absence of priming by fungal hyphomycetes on *A. pseudoplatanus* and *R. ponticum* (Jabiol and Chauvet, 2012, Jabiol et al., 2013). The comparatively low FPOM produced from *A. glutinosa* suggest a greater conditioning by hyphomycetes and assimilation of leaf material by decapods in relation to the other two leaf species. This is in accord with the higher growth rate observed by decapods in the presence of *A.*

glutinosa, in relation to the negligible growth in the presence of *R. ponticum*. However, growth rate was highest for *A. pseudoplatanus* which produced more FPOM, which could be explained by the greater availability of phosphorous in this species compared to *A. glutinosa* (Hladyz et al., 2009).

This study provides an example of an invasive alien species (here decapod crustaceans) providing biotic resistance to the effects of other, problematic species. *R. ponticum* was introduced to the British Isles in the 19th Century where it has become widespread and is a high priority for management (Hill and Hulme, 2012). Its impacts on freshwater ecosystems include provisioning of leaf litter that is of low nutritional quality and thus decomposes at a much lower rate (Hladyz et al., 2011). The presence of large amounts of *Rhododendron* leaf litter that does not decompose is likely to also alter the structural heterogeneity of sediment surfaces. The invasive alien decapod species degraded this leaf litter at a greater rate than the native crayfish, which may in part ameliorate the impact of this alien litter. There was however negligible growth of decapods that consumed this species, suggesting there would be no selective advantage to consuming this material when more nutritious resources are available. Thus, this invasive alien riparian plant does not appear to facilitate these invasive alien decapods.

The Sycamore, *A. pseudoplatanus* is native to mainland Europe with exclusion from the British Isles likely caused by post-Pleistocene isolation before they could be colonized by this tree species. It was first recorded growing outside of planting sites in the British Isles in the 17th Century and is now widespread (Squirrel, 2015). *A. pseudoplatanus* litter have however been studied and have shown similar impacts (decomposition rates, N:P, cellulose, fungal biomass, invertebrate colonization) to other native species, particularly *A. glutinosa* (Abelho, 2001, Hladyz et al., 2009). Thus *A. pseudoplatanus* could be predicted to have lower impacts on native freshwater biodiversity and ecosystem processes than non-European non-native species (Ricciardi and Atkinson, 2004, Paolucci et al., 2013). This is supported by the results of our study where *A. pallipes* consumed and grew following consumption of this leaf species, potentially providing biotic resistance to accumulation of this leaf species. The other two decapod species also consumed and grew in the presence of Sycamore leaf litter.

Biological resistance to the establishment of newly colonizing species, including invasive alien species has been a commonly measured variable of ecosystem functioning (Fargione and Tilman, 2005). Native species providing resistance to the *impacts* of invasive alien species have not been explicitly studied, though native crayfish have been found to reduce establishment of invasive alien snails (Olden et al., 2009, Dorn and Hafsadi, 2016). The lack of processing of *R. ponticum* by the native crayfish *A. pallipes* however shows this native decapod species does not provide a functional resistance to the impacts of this invasive alien shrub, as opposed to invasive alien decapods. The enhanced decomposition and assimilation of native leaf litter by invasive alien decapods in relation to *A. pseudoplatanus* and *R. ponticum* however suggest native leaf litter could be depleted first, removing higher quality resource to other detritivores (Hladyz et al., 2009). This competition for leaf litter could therefore result in further declines of consumers of detrital resources, and could even exacerbate the impacts of *R. ponticum* if decapods prefer other leaf species when given the choice. This could be confirmed by measuring standing stocks of different leaf litter and shredding invertebrates in alien crayfish and riparian tree invaded habitats, as well as mesocosm experiments with leaf litter mixtures, detritivorous invertebrates and decapods. Invasive alien decapods have been shown to remove invasive alien leaf litter, reducing the negative impact of that leaf litter that would be otherwise unavailable to the remainder of the food web. This demonstrates an interaction of multiple invasive alien species leading to a combined, potentially buffering effect on ecosystem functioning. This provides an example of invasive alien species introducing complementarity to biodiversity-ecosystem relationships, whereby addition of a species increases a measured ecosystem process such as transformation and capture of a resource (Tilman, 1999, Cardinale et al., 2002). Invasive alien decapods have already been shown to provide services to their host ecosystems through provision as prey to larger organisms (Lodge, 2012), the current study demonstrating both supporting and regulating services through the recycling of energy and nutrients from leaf litter of pest species. Given these findings, the costs and benefits of management of invasive alien decapods and riparian plants could be developed where management of riparian invaders is prioritised to improve the overall health of freshwater ecosystems and should be taken into account for invasive species management.

Chapter 4. Impacts of native and invasive alien decapods on biodiversity and ecosystem processes

4.1 Introduction

Biological invasions are one of the most widespread and damaging pressures on freshwater ecosystems (Leprieur et al., 2009, Strayer and Dudgeon, 2010, Ricciardi and MacIsaac, 2011). The mechanisms by which invasive alien species influence ecosystems are frequently complex and challenging to predict (Simberloff et al., 2013). A recipient ecosystem might have no similar species to the invader, resulting in novel impacts, or a functionally similar species might be present. In the latter scenario, the invader would either co-exist with the similar native, or it may extirpate it. Redundancy of invasions could then be predicted if the functional traits of the invader are the same as the native (Walker, 1992, Rosenfeld, 2002, Dunoyer et al., 2014, Magoulick and Piercey, 2016). Invasive alien species occur at all trophic levels of freshwater ecosystems, and often include omnivorous consumers such as decapod crustaceans (Gallardo et al., 2015). Invasive alien decapods (crabs and crayfish) are common invaders of freshwater ecosystems globally, causing changes to ecosystem services (Lodge et al., 2012), especially in Western Europe (Keller et al., 2009).

Decapod crustaceans have considerable effects on freshwater ecosystems (Lodge et al., 1994, Parkyn et al., 1997, Usio, 2000, Usio and Townsend, 2002, Dorn and Wojdak, 2004, Rosewarne et al., 2013). They can modify the physical habitat through burrowing, foraging and other behaviours, resulting in increasing turbidity and suspended material (Statzner et al., 2000, Statzner et al., 2002, Harvey et al., 2011, Harvey et al., 2013). Decapods can also change dissolved nutrient concentration in water columns through excretion (Kristiansen and Hessen, 1992, Evans-White & Lamberti, 2005, Chapter 2). Most conspicuous are trophic impacts, with omnivorous decapods consuming: leaf litter (Emmerson and McGwynne, 1992, Usio, 2000, Schofield et al., 2008, Dunoyer et al., 2014); carrion (Gladman et al., 2012); living macrophytes (Nyström and Strand, 2003); algal biofilms (Gherardi and Lazzara, 2006); and invertebrate consumers at many trophic levels (Nyström et al., 1999, Gherardi et al., 2001, Nyström et al., 2001, Stenroth and Nyström, 2003, Crawford et al., 2006, Peay et al., 2009, Haddaway et al., 2012, Rosewarne et al., 2013, Arribas et al., 2014). These trophic impacts can result in cascades leading to increased periphyton production (Nyström et al., 2001, Stenroth and Nyström, 2003, Jackson et al., 2014, Rosewarne et al., 2016) and decreased detritus decomposition (Dunoyer et al., 2014, Lagrue et al., 2014). Invasive alien decapods therefore have the potential to impact freshwater biodiversity and ecosystem processes at multiple trophic levels.

Despite the known impacts of invasive decapods on freshwater ecosystems, the ecological consequences of replacement of native decapods species by non-native species have been infrequently studied: studies have typically been in the context of the decapod becoming introduced to ecosystems naïve to this taxonomic group (e.g. Crawford et al. 2006; Grey & Jackson, 2012; Moore et al. 2012, James et al. 2014). Two meta-analyses of the impacts of crayfish on biodiversity and ecosystem functioning only compared impacts of crayfish upon crayfish-free controls and not to treatments with analogous natives (Gallardo et al., 2015, James et al., 2015). One meta-analysis was able to make use of eight laboratory and enclosure experiments comparing native to invasive alien crayfish, but the majority were in North America, and were limited to measurements of single taxa or trophic levels (algae, macrophytes, snails, other invertebrates, fish or amphibians (Twardochleb et al., 2013) rather than assessing impacts across multiple trophic levels, ecosystem attributes and processes. Laboratory studies that did compare consumption rates of native and invasive crayfish have indicated stronger top-down impacts of invasive alien than native species (Haddaway et al 2012, Dunoyer et al. 2014, Rosewarne et al 2016, Chapter 2). These studies showing this stronger top-down effect have so far not been thoroughly verified in more realistic, replicated scenarios.

Establishing impacts of invasive alien decapods has often involved either laboratory studies (Johnson et al., 2011, Haddaway et al 2012, Harvey et al. 2013, Lagure et al. 2014, Rosewarne et al 2016), observations in the field following the progress of the invasion (Guan and Wiles, 1998, Harvey et al., 2013), use of enclosure (or cage) experiments in the field (Arce et al., 2006, Lagrue et al., 2014) or use of mesocosms (Jackson et al. 2014, Rosewarne et al., 2016). Studies in the field are limited by reduced replication and/or confounding factors such as between site variation in factors such as temperature or disturbance. Enclosure studies can be difficult to interpret as they occur in already impacted sites that unrealistically shift the control baseline, and to conduct them in unimpacted sites risks release of the invasive alien species. Laboratory and small-scale "bottle" studies have the advantage of having greater replication and reduced confounding factors, but are limited by their lack of realism (Parsons, 1982, Schindler, 1998). Field-based mesocosm studies (use of water tanks or flumes) have the advantage of increased complexity than laboratory microcosms and are replicated to avoid confounding factors that disparate field sites might generate (Schindler, 1998, Benton et al., 2007, Harris et al., 2007, Ledger et al., 2009, Stewart et al., 2013). They can be set up to hold multiple trophic levels undergoing ecosystem processes under more natural conditions to verify observations in the field and in the laboratory (Petersen and Hastings, 2001, Ledger et al., 2009, Brown et al., 2011, Stewart et al., 2013). Mesocosms also have the advantage of controls not being impacted *a priori* (such as "ghost of invasion past", *sensu* Kueffer et al., 2013) as in enclosure experiments.

An example of a biological invasion that has the potential to cause significant shifts in food webs and thus functioning of freshwater ecosystems are the American Signal Crayfish (*Pacifastacus leniusculus*) and the Chinese Mitten Crab (*E. sinensis*) into river basins previously dominated by the White Clawed Crayfish (*Austropotamobius pallipes*). *A. pallipes* is classified as "Endangered" by the IUCN, and is threatened primarily by invasive alien crayfish species (Füreder, 2010). *P. leniusculus* extirpates and replaces the native *A. pallipes* through competition (Holdich and Reeve, 1991), and through its role as a vector of crayfish plague-causing *Aphanomyces astaci* (Alderman et al., 1990). Impacts of *E. sinensis* on native crayfish such as *A. pallipes* are unknown, but is proposed to be a threat (Clark et al. (1998) and this species is also known to carry *A. astaci* (Schrimpf et al., 2014). Ecological consequences of replacement of *A. pallipes* by either invasive alien decapod (*P. leniusculus* or *E. sinensis*) are not fully understood.

Laboratory microcosm studies comparing these invasive alien species with native *A. pallipes* have shown differential metabolic (Rosewarne et al., 2014) and resource consumption rates (Edmonds et al., 2011, Haddaway et al 2012, Rosewarne et al. 2016, this study, Chapter 2), which are traits likely to drive changes in the food web even in the unlikely case of a 1:1 replacement of individual natives with invasives. Field observations explicitly assessing effects of *A. pallipes* replacement by *P. leniusculus* (as opposed to impacts of *P. leniusculus* without reference to previous invasions or uninvaded sites) have shown declines in fish recruitment (Peay et al., 2009). Another survey found fewer benthic fish in invaded reaches compared to those with native crayfish (Bubb et al., 2009). No studies exist on replacement of *A. pallipes* by *E. sinensis* possibly due to *P. leniusculus* extirpating the native crayfish before invasion of the crab, and because *A. pallipes* is rarely studied in lower reaches of river basins. In the laboratory, studies comparing *A. pallipes* with *E. sinensis* have shown increased predatory functional responses to macroinvertebrate prey (Rosewarne et al., 2016), and enhanced direct processing of leaf litter, by *E. sinensis* (Chapter 2). However, broader ecological impacts have yet to be assessed and verified in more complex yet replicated experimental venues that control for extraneous variables and confounding factors, with multiple levels of trophic organization. Understanding the outcomes of invasions is necessary for prediction and to justify preventative measures or management of already invaded ecosystems (Strayer, 2010) .

This study aimed to measure the ecological impacts of *P. leniusculus* and *E. sinensis* on lentic freshwater ecosystems, and to compare these impacts to those of native *A. pallipes* to understand the consequences of its replacement by these invasive alien species. Ecological impacts considered include macroinvertebrate densities and community structure; ecosystem functioning such as productivity at lower trophic levels (algal biofilm, phytoplankton and submerged macrophytes), leaf litter decomposition, community respiration and gross primary productivity; and water quality parameters. Specific aims were:

(H_i) To measure the impact on macroinvertebrate community structure. Previous lab studies have revealed the enhanced resource consumption, metabolic and activity rates of invasive alien decapod species (Haddaway et al., 2012, Rosewarne et al., 2014, 2016). It was hypothesised that this should result in increased consumption of resources, especially slow moving species such as gastropods and other invertebrates such as smaller crustacean species.

(H_{ii}) To measure the impact on autogenic ecosystem processes. A higher rate of predation upon grazing macroinvertebrates was expected to reduce grazing pressure and lead to an increase in primary and gross primary production. Increased respiration by invasive decapods was expected reduce net ecosystem productivity via ecosystem respiration.

(H_{iii}) To measure impacts on allochthonous ecosystem processes. Depletion of shredding invertebrate species was also expected to be higher in invasive species treatments. However, enhanced leaf litter breakdown rates by invasive alien decapods have been observed in the laboratory (Moore et al., 2012, Chapter 2), which may result in decomposition rates being maintained or even enhanced.

(H_{iv}) To measure the impact on water quality - invasive alien decapods were expected to alter water quality through increased turbidity from bioturbation (Harvey et al., 2013) and increased particulate and dissolved carbon from the breakdown of leaf litter (Chapter 2, Carvalho et al., 2016); altered dissolved nutrients such as ammonium and nitrates from excretal products from more consumptive invasive alien species (Chapter 2, Evans-White & Lamberti, 2005, Usio et al., 2006). As with ecosystem processes, the deterministic changes induced to the ecosystem by the invasive decapods were expected to decrease the overall variability of the water quality.

4.2 Materials & Methods

4.2.1 Experimental set-up

To investigate the ecological consequences of replacement of native by invasive alien decapod species on lentic freshwater ecosystems, an array of outdoor mesocosms was established (Fig 4.1). These consisted of 16 plastic water tanks 0.65 m deep, 1 m diameter and 0.78 m^2 in area dug into the ground. These were located on the University of Leeds Field Research Unit, Spen Farm near Tadcaster, West Yorkshire. To prevent decapod escape, water tanks were covered with mesh (20 mm aperture), gravel covered the ground between water tanks to suppress weeds and enhance desiccation of escaping invertebrates, and a plastic fence (~0.3 m deep, 1 m high) surrounded the array.

Figure 4.1. Water tank mesocosm facility used for the experiment on impacts of invasive alien decapod species in this chapter. Shown also is the array of dissolved oxygen probes and data loggers measuring community respiration and an anemometer measuring wind speed to correct for reaeration.

Each mesocosm was filled with water from a nearby borehole. A substrate of a 9:1 mix of sand and Aquasoil and gravel of ~ 3 cm depth was then added to each mesocosm. Microbial communities were added (09/04/2013 – 73 days prior to the experiment) through the addition of 1 litre inoculum from a homogenate of water originating from a broad range of freshwater habitats. The latter were so far not invaded by alien decapods: a stream (Meanwood Beck, Leeds); a canal (Leeds-Liverpool canal); three ponds (Woodhouse Moor, Woodhouse Ridge, Hollybush Centre-Leeds); and a lake (Wothersome Lake, Bramham estate). Native macrophytes in the pond consisted of *Ceratophyllum demersum*, 115 g wet mass rinsed with deionised water to remove large numbers of passenger invertebrates, plankton and microbes. This species was chosen because it is abundant in lentic freshwaters in the UK and Europe (Duigan et al., 2007), and also because it had previously survived successfully in these water tanks in previous experiments (Rosewarne et al., 2013). These were planted in the middle of the water tank (11/04/2013 – 72 days prior to the experiment) in pots containing gravel and 1 ml of pond plant compost (Aquasol®). To allow a standard surface for the sampling of algal biofilm, an unglazed tile was placed on the sediment surface of each mesocosm so it would receive the same light exposure. To provide a resource for the a "brown" compartment of the food web, leaf litter detritus was added in the form of 10g Alder (*Alnus glutinosa*) and 40g Oak (*Quercus rober*) which had been air dried following abscission before weighing. In addition, three packs of pre-weighed (5 g) oven dried Alder leaves with coarse mesh (20 mm aperture) were added to each mesocosm to enable decomposition rates to be estimated. These were accompanied with a single fine pack (0.5 mm aperture, to exclude most macroinvertebrate shredders) of Alder leaves to differentiate microbial from macroinvertebrate break down. Macroinvertebrates with limited colonization abilities were added to each mesocosm 30 days prior to addition of decapods to the experiment: chironomid larvae (*Chironomus* sp. ~ 1500 individuals); *Gammarus pulex* (100 individuals); *Asellus aquaticus* (100 individuals); *Baetis rhodani* (50 individuals); Cased-caddis larvae, *Sericostoma* sp. (10 individuals); and six species of snail (100 individuals of *Radix peregra*, 10 *Planorbis corneus*, 10 *Lymnaea stagnalis*, 10 *Lymnaea palustris*, 20 *Physa fontinalis*, 10 *Bithynia tentaculata*). Other invertebrates colonised naturally (listed in Supplemental Table 4.1).

Collectively, the water tanks held 47 taxa of 12075 individuals. Taxa added to the mesocosms that did not consistently persist included *G. pulex* and *B. rhodani*, found in only a few mesocosms (regardless of treatment) and in low numbers in the case of *Gammarus* or in only two mesocosms in the case of *Baetis*. Tricopterans (both seeded and colonised) were present, but represented by only singletons, with empty cases or pupae found, suggesting the majority had pupated during the study. The mean most dominant species (by percentage of individuals) were gastropods (39%), chironomid larvae (37%) and *A. aquaticus* (13%). Odonata larvae occurred in five of the mesocosms, but represented 1-6 individuals of one species per mesocosm of either Coenagrionidae, *Platycemis* sp., or Libellulidae. Coleoptera showed moderate abundance, but contributed the most species to the assemblage (30%) after gastropods (33%). The water tanks were also colonised by water boatmen (*Micronecta* sp. and *Notonecta* sp.). A full list of invertebrate species occurring in the ponds and their origin is listed in Supplemental Table 4.1. General water quality parameters are given in Supplemental Table 4.2.

For the decapod treatments, White Clawed Crayfish were collected by hand at Porter Brook, Sheffield (latitude: 53.360970, longitude: -1.5445179), following the draw-down of the stream to collect these crayfish for a translocation organized by the Environment Agency and PBA Ecology and licensed by Natural England (licence # 20122661). The American Signal Crayfish were collected from Loch Ken, Dumfriesshire (latitude: 55.025464, longitude: -4.0829659). Chinese Mitten Crabs were collected from the River Thames at Chiswick Mall (latitude: 51.488489, longitude: -0.24471175). All decapods were held > 3 months in the laboratory to allow acclimatisation, and introduced to the mesocosms following 24 hours of no feeding.

4.2.2 Experimental design

The experiment ran from 21st June when the decapods were added, to 22nd July 2013 (33 days total). Treatments were arranged in a randomised factorial design, with four replicates each of native crayfish (*A. pallipes*), invasive alien crayfish (*P. leniusculus*), invasive alien crab (*E.*

sinensis) treatments, and a control with no decapods. Each mesocosm (with the exception of the controls) had two sub-adult decapods (carapace length: 31.78 ±3.07 mm for White Clawed Crayfish; 34.08 ± 2.31 mm for American Signal Crayfish; 29.37 ± 3.56 mm for Chinese Mitten Crabs), giving a density of 2.56 m^{-2}. These densities were generally within the range of those observed in field sites (Nyström, 2002, Rudnick et al., 2003).

4.2.2.1 Macroinvertebrate sampling

At the end of the experiment, invertebrates were sampled using a hand-net (250 µm aperture mesh) swept repeatedly through the pond water for one minute , including scraping the sides of the water tank and disturbing the sediment surface. While pond surveys often consist of three minutes to sample using a net (Nicolet et al., 2004), macrophytes and leaf packs had already been removed to sample invertebrates and the compact size of the ponds enabled complete sampling to be achieved in one minute. Debris from the nets was placed in labelled plastic zip lock bags and treated with ~ 70% EtOH solution to kill and preserve invertebrates. Invertebrates from these samples were later sorted from debris in the laboratory, identified to species level where possible (using Dobson et al., 2012 and references therein) and enumerated. Invertebrates from macrophytes and leaf packs were counted separately to the general benthic-pelagic sample to assess whether there were any habitat-specific patterns in their distribution in the presence/absence of various decapods. Zooplankton (*Daphnia*, ostracods & copepods) were captured using these sampling techniques, but they were not included in the final analysis as the methods used were not appropriate for this size of organism.

4.2.2.2 Ecosystem functioning

Leaf packs were removed at the end of the experiment, rinsed with deionised water and oven dried at 50^{o}C to constant mass, weighed then ashed at 500^{o}C to calculate ash-free dry mass (AFDM). Decomposition rates were calculated as the change in estimated AFDM following (Benfield, 2006). FPOM in the water column & benthos was sampled using suction from a 20 cm diameter, 70 cm cylindrical tube, stored in a plastic sample bag and treated with methylated spirit solution for later processing. These samples were filtered first through 10mm mesh, then through 0.7 µm GF/F filter discs, oven dried at 105^{o}C for 24 hours, weighed following cooling, then ashed at 500^{o}C to calculate AFDM.

Macrophytes were removed and stored in plastic zip lock bags and either frozen and/or supplemented with ethanol to kill and preserve invertebrates and plant matter. These were rinsed in deionised water to remove invertebrates (that were stored in ~ 70% EtOH) and other material, and dried for 24 hours or until constant mass at 50^{o}C, then ashed at 500^{o}C to estimate AFDM. Filamentous algae abundant in all the ponds but not measured due to time constraints and the difficulty in separating other matter to obtain reliable estimates of mass.

Biofilms (bacteria, fungi and periphyton) were sampled from the tiles using a nylon brush. Primary productivity (measured as chlorophyll *a*) of periphyton from a 5 ml sub-sample was measured using the filtration and spectrometric method of Steinman et al. (1996). Biofilm biomass was measured as for FPOM described above. Water samples were collected from the surface water for plankton, and a 5ml sub-sample measured for chlorophyll *a* and biomass measured as for periphyton.

Community respiration and gross primary productivity of the mesocosms was measured 30 days into the experiment using the diel oxygen technique, where probes recorded changes in dissolved oxygen (D.O.) every 15 minutes for 24 hours using a YSI Environmental ProODO^{TM} logger, following Bott (1996). The premise of this technique is that changes in D.O. are linked to the extent of both photosynthesis and respiration by the community, with daily respiration estimated from the change in oxygen in darkness (reduction of photosynthetically active radiation verified using a Delta QS-5 quantum sensor connected to a Campbell® CR800 data logger). D.O. was corrected for re-aeration from wind-induced gas exchange with the surface water and atmosphere by measuring wind speed (using an anemometer connected to a Campbell® CR800 data logger) every 15 minutes, *sensu* Staehr et al. (2010). From these measurements, community respiration (µg O_2 m^{-2} d^{-1}), gross primary productivity (µg O_2 m^{-2} d^{-1}), and net primary productivity (µg O_2 m^{-2} d^{-1}) were calculated.

Sediment respiration was measured following Doering et al. (2011) *in-situ* in enclosed chambers using O_2 consumption over time, assuming equal day and night respiration (Jones et al., 1995, Naegeli and Uehlinger, 1997, Hill et al., 2002). 350 ml opaque tubes measuring 3.8 cm by 34.8 cm (width/length) were bunged with rubber stoppers to create a closed environment (Bott et al., 1985). Bed-surface sediments within the experimental ponds were <8.0mm and this sediment was placed in the tubes for SR calculation (Logue et al., 2004, Doering et al., 2011, Freimann et al., 2013). Tubes were half filled with sediment and then filled with pond water ensuring no head space (Hill et al., 2000, Hill et al., 2002). Tubes were inverted three times, to remove air trapped in the sediment, before measuring dissolved oxygen (D.O.) (Logue et al., 2004). A YSI Environmental ProODO^{TM}, calibrated using a water-saturated air method, was used to measure DO at the start and end of the incubation period (Acuna et al., 2004). D.O. concentrations measured quickly preventing diffusion of atmospheric oxygen into water samples (Logue et al., 2004). Final sediment respiration was calculated following Doering et al. (2011).

4.2.2.3 Water quality

Temperature and dissolved oxygen were measured on day 30 using the probes when using the diel technique mentioned above. Other physiochemical measurements included pH (using a Hanna pH meter) and turbidity (using a Model 2100 A Turbidmeter, CAMLAB, Cambridge). Analysis of water samples collected on day 33 were subsequently analysed with a Skaler SAN ++ continuous flow auto-analyser for ammoniacal nitrogen (NH_3-N), nitrates (N-NO_3 & N-NO_2) and soluble reactive phosphorus (PO_4-P).

4.2.3 Data analysis

Macroinvertebrate parameters calculated included densities (per m^2 of pond) of known decapod prey (snails, isopods and chironomid larvae), taxonomic richness and Shannon diversity. β diversity within treatments was calculated using Jaccard's Index of similarities and compared between treatments. The remainder of statistics were analysed using R (R v.3.1.0.; R Development Core Team, 2014). To contrast macroinvertebrate community structure between treatments, square root transformed invertebrate abundance data were subjected to ordination analysis using non-metric dimensional scaling (NMDS) plots of Bray-Curtis dissimilarities (formula: metaMDS, library: vegan) to view dispersion of similarities, and using PERMANOVA (formula: adonis, library: vegan) to test significance between treatments. This analysis was applied to both the overall community and the subset of species inhabiting the benthic microhabitats (more easily accessible to decapods). Generalized linear models (formula: glm) were used to compare differences between treatments for all response variables (invertebrates, ecosystem functioning and water quality), with the data family selected based on maximum likelihood estimates (library: MASS, formula: fitdistr) and *post hoc* Tukey tests applied (library: multcomp, formula: glht).

Biofilm primary productivity was found to vary with treatment based on the glm described above. Therefore to differentiate causality *post hoc* of top-down regulation of grazing snails by decapods from bottom-up factors such as turbidity, glms were used with primary productivity as a response with treatment as a factor with snail abundance, turbidity, and nitrates as covariates. Because the quasipoisson data family does not produce Akaike's Information Criterion (AIC) values to estimate the best performing glm, Gaussian data family had to be used.

4.3 Results

4.3.1 Macroinvertebrate diversity and abundance

Macroinvertebrate taxonomic richness was significantly lower in the presence of *A. pallipes* compared to the control in the benthic zone of the mesocosms, but not between the controls and the invasive decapods. Shannon index did not differ significantly between treatments overall, or in the benthic zone (Table 4.1). Community ordination (NMDS) plots showed more dispersed community structure in both invasive treatments compared to control or native crayfish treatments, also with less overlap (Figure 4.2) but there was no difference in community structure (Table 4.2). However, beta diversity was significantly greater for *E. sinensis* compared to controls and *A. pallipes* in the benthos, but only between *E. sinensis* and controls for the whole mesocosm sample (Table 4.1, Fig. 4.3c). There was no difference between treatment in the overall richness and density of key decapod prey (gastropods, *A. aquaticus* and Chironomidae). However, when benthic samples were considered separately, gastropods were significantly less abundant in the invasive decapod species treatments compared to the control and to the native crayfish treatments (Figure 4.3, Table 4.1).

Table 4.1. Generalized linear models for macroinvertebrate biodiversity metrics with experimental treatment as the factor

Response variable	**Habitat sampled**	**df**	**Residual deviance**	**Pr(>Chi)**
Taxonomic richness	Overall	3	63.18	0.54
	Benthos	3	14.64	**0.05**
# Individuals	Overall	3	426239.00	0.43
	Benthos	3	92826.00	0.84
Shannon index	Overall	3	0.22	0.28
	Benthos	3	1.36	0.31
β diversity (Jaccard)	Overall	3	0.13	**0.03**
	Benthos	3	0.15	**0.04**
Gastropod sp. richness	Overall	3	0.68	0.51
	Benthos	3	1.51	0.31
Gastropod density	Overall	3	91181.00	0.37
	Benthos	3	27012.00	**0.01**
A. aquaticus density	Overall	3	97.54	0.48
	Benthos	3	26.12	0.88
Chironomidae density	Overall	3	133296.00	0.58
	Benthos	3	277.04	0.75

Table 4.2. Macroinvertebrate community analysis using PERMANOVA

Term	**Degrees of freedom**	**SS**	**Mean SS**	**F. Model**	**R^2**	**P-value (>F)**
(Total community)						
Treatment	3	0.23	0.08	0.81	0.17	0.69
Residuals	12	1.12	0.09		0.83	
Total	15	1.35			1.00	
(Benthic community)						
Treatment	3	0.27	0.09	0.89	0.18	0.59
Residuals	12	1.22	0.10		0.82	
Total	15	1.49			1.00	

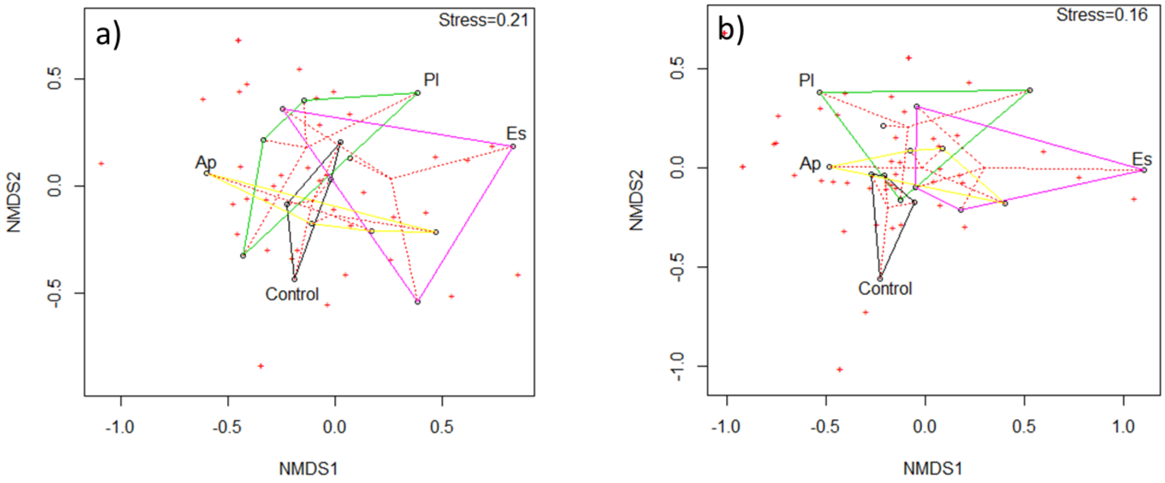

Figure 4.2. Non-metric dimensional scaling (NMDS) plots showing a) the total invertebrate community of the mesocosm and b) invertebrate community from the benthic zone of the mesocosm. Abbreviations for treatments: Ap – *Austropotamobius pallipes*, *Pl* – *Pacifastacus leniusculus*, *Es* – *Eriocheir sinensis*.

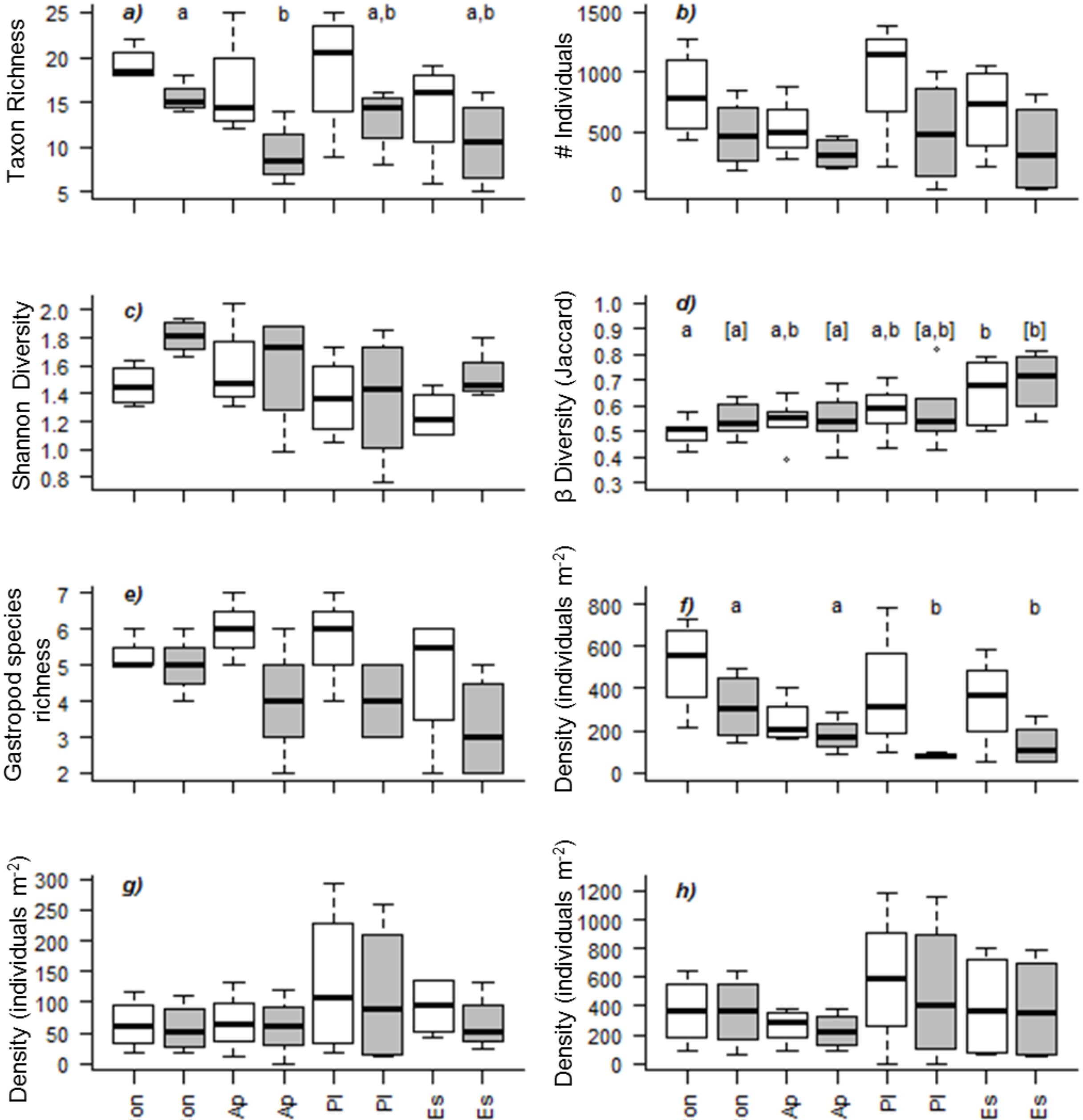

Figure 4.3. Macroinvertebrate community: (a) Invertebrate taxonomic richness; (b) the number of individuals of all invertebrate species; (c) Shannon diversity; (d) β diversity based on Jaccard's Index with letters showing groupings from *post hoc* test, the ones in parentheses for the benthic habitat invertebrate sample; (e) richness of gastropod species; (f) density of gastropods with letters showing groupings from *post hoc* test; (g) density of *Asellus aquaticus*; and (h) density of Chironomidae larvae. White bars show densities for all habitats within mesocosms, grey bars show benthic habitats. Abbreviations of x-axis are: Con – control treatments; *Ap – Austropotamobius pallipes*, *Pl – Pacifastacus leniusculus*; *Es – Eriocheir sinensis*.

4.3.2 Ecosystem functioning

All ecosystem process variables measured showed no significant difference between treatments, with the exception of primary productivity (chlorophyll *a*) of algal biofilms (Table 4.3, Figs 4.4-4.6). Biofilm primary productivity was higher for Signal crayfish treatments than *A. pallipes* and *E. sinensis,* but similar to controls (Table 4.3, Fig 4.5b). These between treatment differences were however not statistically significant based on the *post-hoc* tests. Subsequent modelling found that the interaction between decapod treatment and the abundance of gastropods was a significant factor influencing primary productivity of biofilms, provided the strongest model (Table 4.4). The interaction of treatment and turbidity showed a near significant (p = 0.054) relationship to biofilm productivity, but not the interaction between turbidity and gastropods (Table 4.4). Primary productivity of algal biofilms showed a significant, positive relationship with gross primary productivity (glm p = 0.003, Pearson's correlation R^2=0.61, Appendix 2), and a similar pattern of gross primary productivity (GPP) to periphyton primary productivity was observed between the treatments though not significant. Other primary producers (phytoplankton and submerged macrophytes) showed no significant relationship with GPP (glm p > 0.05).

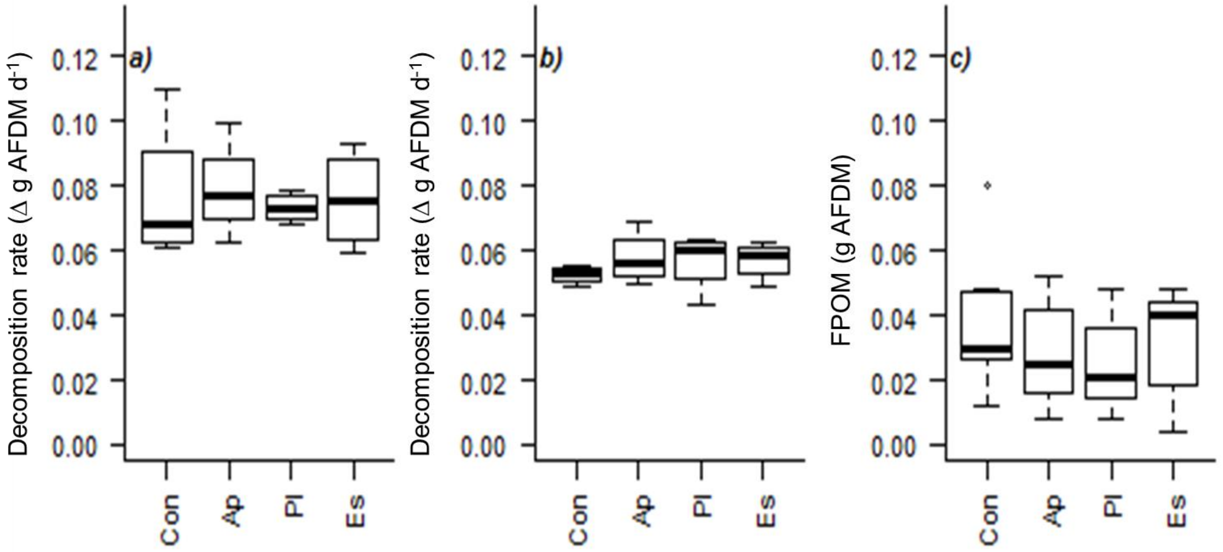

Figure 4.4. Detrital processing in mesocosms: (a) decomposition of leaf litter from macroinvertebrates; and (b) microbial conditioning (decomposition of litter when macroinvertebrates excluded); (c) production of benthic fine particulate organic matter. Abbreviations of x-axis as for Fig 4.3.

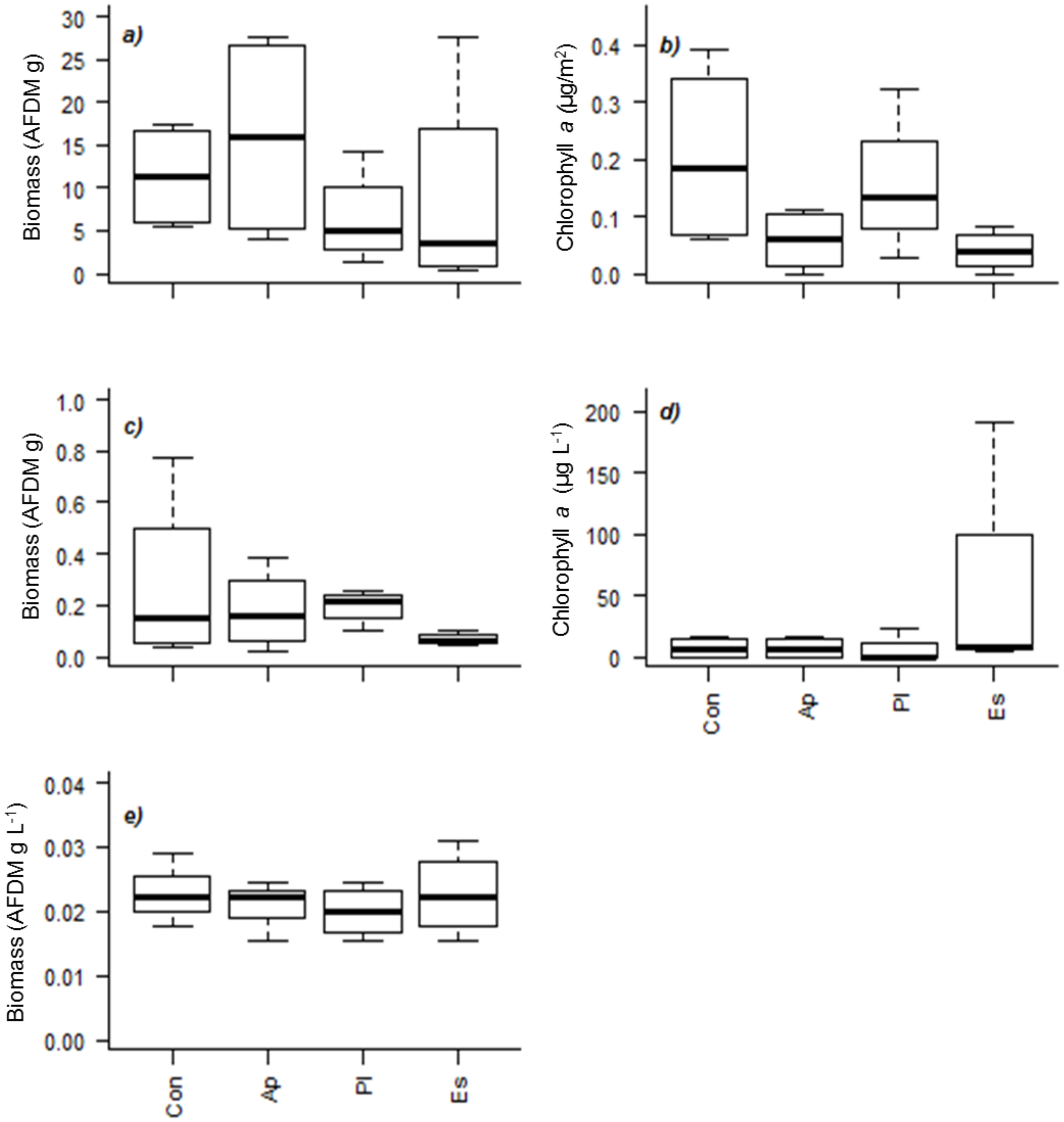

Figure 4.5. Primary production in mesocosms: a) biomass of submerged macrophytes; b) primary productivity of periphyton; c) biomass of biofilms; d) primary productivity of phytoplankton; e) biomass of plankton. Abbreviations of x-axis as for Fig 4.3.

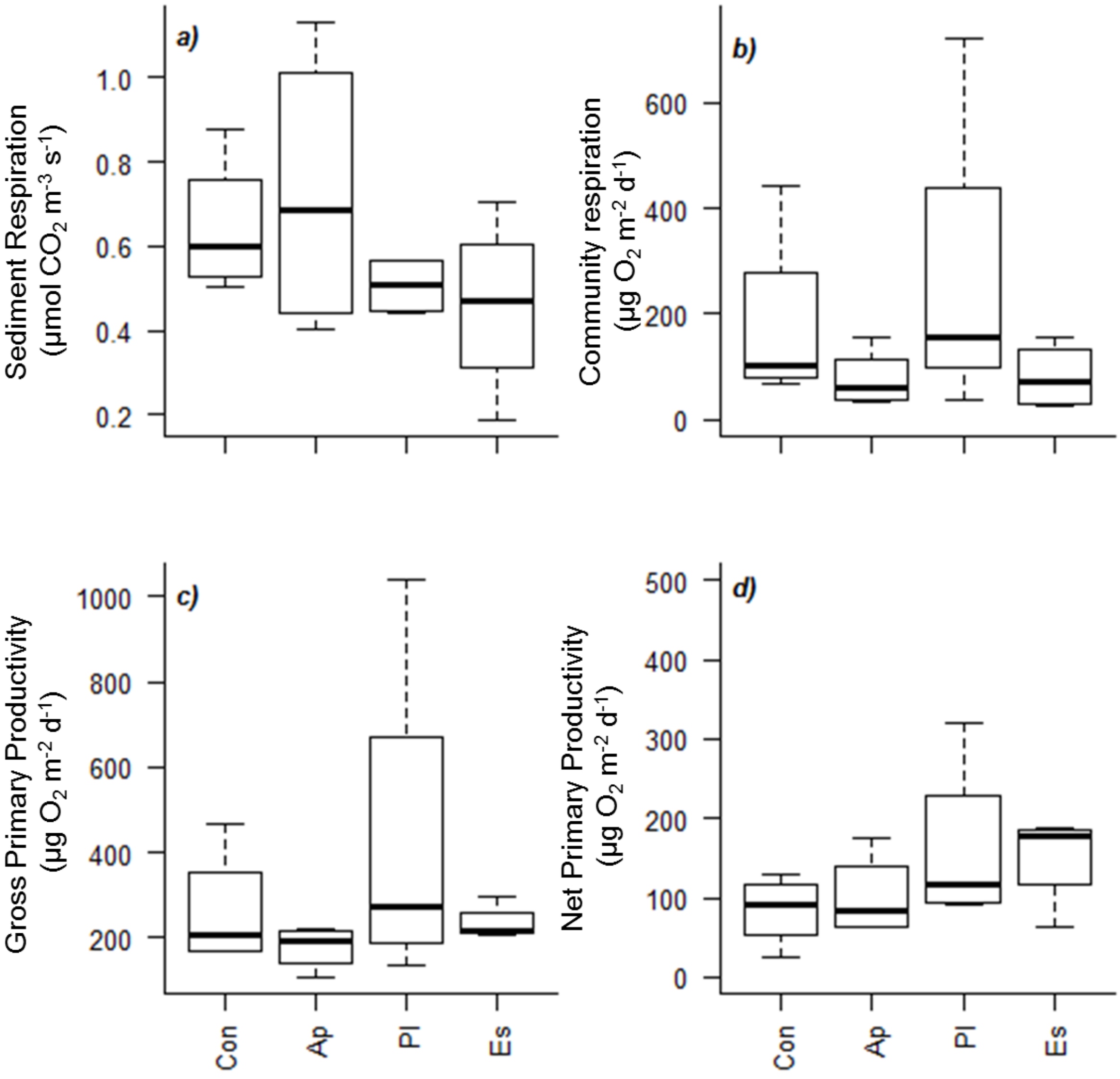

Figure 4.6. Ecosystem-scale respiration and productivity: a) Sediment respiration; b) community respiration; c) gross primary productivity (μg O_2 m^{-2} d$^{-1)}$); d) net ecosystem productivity (μg O_2 m^{-2} d^{-1}). Abbreviations of x-axis as for Fig 4.3.

4.3.3 Water quality

Turbidity differed significantly between the treatments and was higher in the invasive decapod treatments, particularly *E. sinensis* in relation to the control, while *A. pallipes* treatments had a lower mean turbidity compared to the control (Fig 4.7a, Table 4.3). *Post-hoc* tests were not statistically significant between-treatment differences, with only Control-*Es* treatments showing a near-significant comparison (*p* = 0.09). Nitrate differed between treatments (*p* = 0.02) and was highest in native crayfish treatments compared to the control, with *E. sinensis* treatments also high, and *P. leniusculus* slightly higher than controls (Fig 4.7d). These between-treatment differences were however not statistically significant based on the *post-hoc* tests. pH was marginally significant (*p* = 0.05), being lower in all decapod treatments, with pH lower in all decapod treatments and *post-hoc* tests grouping *Ap* and *Es*, similar to the pattern for nitrate. pH had a very significant and positively strong relationship with nitrate concentration (GLM *p*=0.0002, Pearson's correlation R^2= -0.70, Appendix 4.3). The other water quality parameters did not differ significantly with experimental treatments.

Table 4.3. Generalized linear models for ecosystem functioning and water quality with experimental treatment as the factor

Hypothesis	**Response variable**	**df**	**Residual deviance**	**Pr(>Chi)**
Ecosystem functioning	Macroinvertebrate shredding	3	0.01	0.27
	Microbial decomposition	3	0.00	0.68
	Plant biomass	3	198.97	0.55
	Primary productivity biofilm	3	0.68	**0.03**
	Biofilm biomass accrual	3	0.00	0.27
	Primary productivity phytoplankton	3	6441.10	0.40
	Biomass of plankton	3	0.00	0.81
	Sediment respiration	3	0.00	0.26
	Community respiration	3	98932.00	0.39
	Gross Primary Productivity	3	139674.00	0.41
	Net Ecosystem Productivity	3	16709.00	0.34
Water quality	Turbidity	3	10.76	**0.01**
	pH	3	0.49	0.05
	NH_3-N	3	0.37	0.09
	NO_3-N	3	11.05	**0.02**
	PO_4-P	3	0.04	0.14
	Dissolved oxygen	3	12.99	0.23

Table 4.4. Generalized linear models assessing interactions between top-down and bottom-up regulation of algal biofilms

Response variable	**Factor**	**df**	**Residual deviance**	**Pr(>Chi)**	**AIC**
Biofilm primary productivity	Decapod species	3	0.07	0.09	-20
	Gastropod abundance	1	0.00	0.67	-17
	Nitrate	1	0.01	0.38	-18
	Turbidity	1	0.02	0.19	-19
	Decapod*Gastropod	3	0.05	**0.03**	-30
	Decapod*Nitrate	3	0.01	0.92	-13
	Decapod*Turbidity	3	0.06	**0.05**	-24
	Gastropod*Nitrate	1	0.00	0.80	-14
	Gastropod*Turbidity	1	0.00	0.76	-15

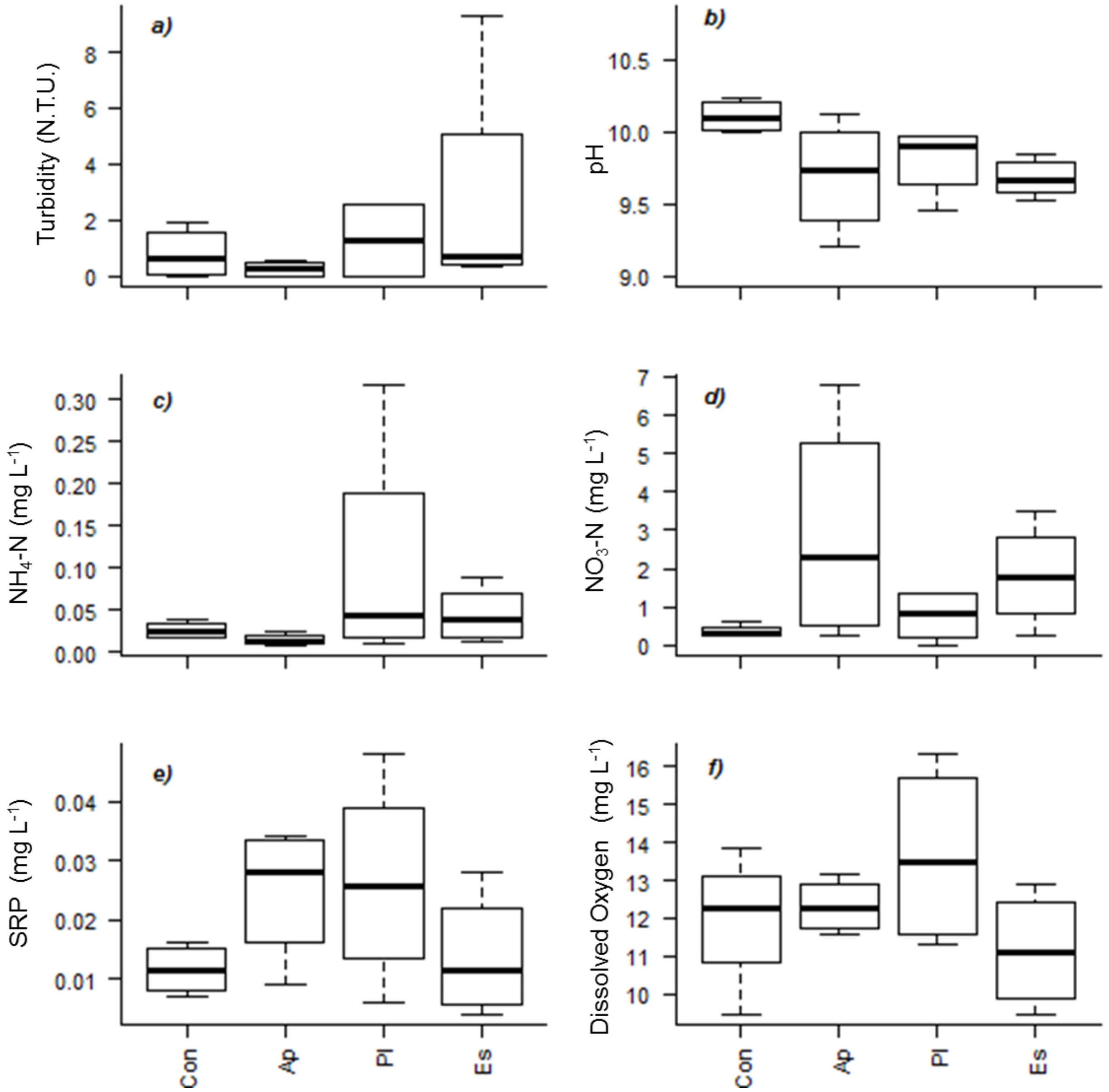

Figure 4.7. Water quality of mesocosms: (a) turbidity; (b) pH; (c) ammonium (NH_3-N); (d) nitrate (NO_3-N); (e) soluble reactive phosphorus (PO_4-P); (f) dissolved oxygen. Abbreviations of x-axis as with Fig 4.3.

4.4 Discussion

To understand the ecological consequences of the replacement of native *A. pallipes* with *P. leniusculus* and *E. sinensis*, an array of replicated water tank mesocosms were randomly assigned to each species, with decapod-free controls. Final diversity and abundances of macroinvertebrates were measured, and with this data ecosystem functioning, and water quality variables were measured and interpreted. This study is novel in that it uses a mesocosm approach to directly compare impacts of the invasive alien species *P. leniusculus* and *E. sinensis* relative to the native species *A. pallipes* that is expected to be replaced.

4.4.1 Impacts on macroinvertebrate community

Invertebrate communities as a whole (taxonomic richness, Shannon diversity, structure based on dissimilarity) did not respond to invasive alien decapods in the experimental water tank mesocosms. However, snail densities in the benthic zone were reduced by invasive alien decapods, while β diversity was higher for *E. sinensis*. Stronger impacts might have been detected had the time period of the experiment been longer than 33 days for the decapods to consume more snails and to physically alter the habitats of the mesocosm through bioturbation. The mesocosms in this study did not consistently sustain amphipod, leech or ephemeropteran populations that have been found to decline in the presence of decapods in other laboratory, mesocosm and field studies (Stenroth & Nyström, 2003, Crawford et al., 2006, Haddaway et al., 2012, Rosewarne et al., 2013, 2016, Mathers et al., 2016). This suggests that effects on invertebrate communities might be stronger had the mesocosms supported more species vulnerable to decapod predation.

The reduced taxonomic richness in habitats accessed by *A. pallipes* was in contrast to Rosewarne et al. (2013) who observed no reduction in response to the presence of this native species. The negligible impacts on taxonomic richness and diversity in response to invasive alien decapods are in accord with Rosewarne et al. (2016) but contrast with the findings of Jackson et al. (2014)

who found a significant reduction of taxonomic richness in mesocosms with *P. leniusculus*. There have been examples of decreases in invertebrate species richness and Shannon diversity in rivers and lakes invaded by *P. leniusculus* (Stenroth and Nyström, 2003, Crawford et al., 2006, Ercoli et al., 2015), possibly reflecting higher densities of invasive versus natives in the field. Increased consumption of invertebrates by *E. sinensis* has been demonstrated in other mesocosm studies (Rudnick and Resh, 2005, Rosewarne et al., 2016), but this is the first study to directly compare the impact of *E. sinensis* with native crayfish in mesocosms, showing slight increases in gastropod predation and greater taxonomic richness and β diversity for *E. sinensis* treatments.

Hypothesised deterministic assembly driven by enhanced predation, consumption of detritus and ecosystem engineering by invasive alien decapods was not clearly observed in the macroinvertebrate community. Visible differentiation of non-metric dimensional scaling was observed for the two invasive species treatments, but this was not significant. However, β diversity measured as Jaccard similarity was greater for *E. sinensis* compared to controls and *A. pallipes*, suggesting more deterministic community assembly in the presence of this invasive alien species (*sensu* Chase et al., 2009). These results contrast to field observations of *P. leniusculus* invasions (compared to the uninvaded, crayfish-free sites), where invertebrate community structure differed considerably between invaded and un-invaded sites (Crawford et al., 2006, Jackson et al., 2014, Mathers et al 2016).

The reduced snail abundance in invasive alien species treatments in the benthic zone (which had the most access by decapods) was as expected from laboratory studies that showed higher rates of predation by the invasive than the native species on snail prey (Haddaway et al., 2012, Taylor & Dunn, unpublished) and previous mesocosm studies of these two invasive decapod species also show similar impacts on gastropods (Rosewarne et al., 2016) or other benthic mollusc species (Rudnik & Resh, 2005). However, there was no overall decline of gastropods, with snails persisting on plants apparently out of range to foraging decapods. The negligible difference in chironomid abundance between native, invasive and control treatments was however not expected, as previous studies demonstrated higher predation of chironomids by invasive alien decapods (Haddaway et al., 2012, Jackson et al., 2014, Rosewarne et al. 2016). Laboratory studies on crayfish-prey interactions have found increased abundance of prey species in microhabitats with limited access to crayfish (Dunoyer et al., 2014). It is likely that the habitat complexity of the mesocosms in the current study permitted co-existence of vulnerable prey species with invasive alien decapod species. This demonstrates that inferences from scaling from homogenous lab experiments to real-world heterogeneous environments are likely to be limited. It also highlights the habitat-specific impacts invasive species have on freshwater ecosystems (*sensu* Strayer, 2010), in this case decapods impact benthic invertebrate community. The consequences of replacement of *A. pallipes* by *P. leniusculus* and/or *E. sinensis* at equivalent densities in the field would thus be expected to cause reduced gastropod densities in the benthos, and this impact will likely be amplified by increased densities of these invaders, as observed in other alien crayfish invasions (Charlebois and Lamberti, 1996). The mechanisms of this impact remains uncertain as to whether they are driven by greater variability in feeding preference within each invasive alien species, by habitat modification through bioturbation (see below), or a combination of the two.

4.4.2 Impacts on ecosystem functioning

Despite enhanced consumption of leaf litter by *P. leniusculus* and *E. sinensis* in the laboratory (Chapter 2), leaf packs in the mesocosm showed no greater decomposition or derived secondary products (FPOM) in the presence of decapods in relation to the control. This is in contrast to laboratory studies (Chapter 2, Montemarano et al., 2005, Dunoyer et al., 2014) but in agreement with mesocosm studies for these species (Rosewarne et al. 2016). A reduction in decomposition was expected as a result of decapod predation on key shredders such as *A. aquaticus*. However, surprisingly there was no significant difference in shredder abundance between different treatments. The lack of difference in decomposition also suggests no difference in direct shredding by the three decapod species in the mesocosms. This demonstrates a possible limitation of laboratory studies, which have shown enhanced decomposition by decapods (e.g. Chapter 2), where heterogeneity of resource availability was greatly simplified compared to the mesocosm.

The only abundant shredding macroinvertebrate species present in the mesocosms was *Asellus aquaticus,* which is an inferior decomposer of leaf litter compared to other shredding species (McKie et al., 2008), and did not show any difference in abundance between treatments. The poor survivorship in the mesocosms of a high performing shredding species, *Gammarus pulex* (MacNeil et al., 2010a, Piscart et al., 2011) prevented the opportunity to assess the impacts of decapod regulation of this species by decapods, and cascading effects on leaf litter breakdown.

The short-term nature of the experiment may have concealed both the climax community (or alternative stable state) of each pond mesocosm for each treatment, resulting in the general neutral impacts observed. For example, leaf litter breakdown rates might have reflected those in the laboratory once the most vulnerable species were consumed and diminished by the decapods. The experiment took place during summer months, where populations of *Gammarus* could have been more diminished by heat, as these water tank mesocosms have been observed to hold numerous individuals of this species during later summer when the temperature would have been lower (Rosewarne et al 2013, 2016).

Primary productivity of periphyton (algal biofilms), which strongly influenced the gross primary productivity of the mesocosms, was higher for *P. leniusculus* compared to *A. pallipes* and *E. sinensis* treatments. Despite experimental treatments interacting with turbidity to influence primary productivity, the trophic cascade appeared to be controlled by the top-down impact of decapod predation on snails. This is consistent with trophic cascades driven by grazer predation by invasive alien decapods (Jackson et al 2014, Gallardo et al 2015, Rosewarne et al., 2016). This study has shown an invasive alien crayfish influences this trophic cascade to a greater extent than the native crayfish it replaces.

Plankton biomass and primary productivity were not affected by either decapod treatment, explained by increased decapod activity in benthic as opposed to pelagic habitats of the ecosystem (Strayer, 2010). Biomass of the submerged macrophyte (*Ceratophyllum*) was expected to be reduced compared to controls and native crayfish treatments through direct consumption or shredding by omnivorous decapods, and while this pattern was observed it was not significantly different to controls (Fig 3d). This absence of impact on macrophytes has also been observed by Rosewarne et al. (2016), but other studies have found depletion of macrophytes by *P. leniusculus* (Nyström et al., 1996, Nyström et al., 2001, Nyström and Strand, 2003). The presence of more nutritious food items, such as animal prey is likely to be a factor in this observation (Magoulick and Piercey, 2016).

The hypothesised (H_{ii}) increase in community respiration did not occur. This was potentially due to depletion of prey by decapods that would contribute to the overall respiration – oxygen that would have been consumed by depredated snails could otherwise be consumed by the decapod predators. Variation in species composition is known to have variable effects on community respiration, including neutral effects (Downing and Leibold, 2002). This relationship could be verified through obtaining respiration rates from individual prey invertebrates and assess whether their change in abundance at the expense of the invasion of a decapod would be equivalent gain in respiration rate. Increased bioturbation might have caused increased biological mixing depth of sediment and thus increased sediment respiration, but this was not observed and contributions from other invertebrates to bioturbation could also be a factor (e.g. Navel et al., 2012). Despite its significant correlation to primary productivity of biofilms (partly explained by increased predation of snails), gross primary productivity (GPP) was also not significantly affected by invasive decapods. A greater density of decapods could induce an increase in GPP through increased predation pressure on snails (Charlebois and Lamberti, 1996) driving increased biofilm and macrophyte production and could be verified through further experimentation.

4.4.3 Impacts on water quality

This is one of the first comparisons of the effect of *A. pallipes* and *E. sinensis* on water turbidity, with previous studies so far examining only *P. leniusculus* (Johnson et al., 2011, Harvey et al., 2013). Turbidity of the water column was found to be higher in invasive alien decapod treatments than in controls or even native decapod treatments. Suspended sediment was not measured, but the increased turbidity in invasive decapod treatments in the absence of correlates with phytoplankton production indicates increased suspended sediment via bioturbation was the most likely cause. As surface waters were sampled, turbidity measurements nearer the sediment bed are likely to have been more pronounced: rivers invaded by *Pl* also show increased turbidity at bed flow compared to mid flow (Harvey et al., 2013). Crayfish and other decapods increase sediment transport through burrowing, foraging, conspecific antagonistic interactions and general movement across the sediment (Harvey et al., 2011, Johnson et al., 2011) and increased burrow density and structure (Rudnick et al., 2005). Increased turbidity at surface waters for

Mitten Crab treatments indicates a very high impact by this species in relation to uninvaded ecosystems.

The increased nitrate concentration in decapod treatments suggests nitrification of ammonia excreted by decapods. Isolation of these species in the laboratory has shown a similar pattern of ammoniacal nitrogen (NH_3-N) production for each species, with similar values for *A. pallipes* and *E. sinensis* and lower values for *P. leniusculus* (see Chapter 2). The lower ammonia excretion by *P. leniusculus*, leading to lower NO_3–N production suggests this species is likely to be a greater nutrient sink (*sensu* Vanni, 2002) compared to *A. pallipes*, through consuming and assimilating more nitrogen and reducing its availability to other organisms (particularly absorbing species such as primary producers). This suggests that invasions by *Pl* and the extirpation of *Ap* could affect nitrogen cycling and community structure in N-limited ecosystems. A similar relationship has been observed for the replacement of the native Japanese crayfish *Cambaroides japonicus* by *P. leniusculus* (Usio et al., 2006).

Other water quality parameters, such as dissolved oxygen, pH, and nutrients were expected to differ due to changes to community respiration, invertebrates, detritus and autotrophs through consumption, trophic cascades and bioturbation. Changes to pH were predicted based on differences in nutrient excretion, and while there were differences between treatments, these were not significant. These parameters are commonly measured during monitoring of water quality, and it is likely that extirpation of native crayfish with or without replacement by invasive analogues will alter at least some of these.

It may be concluded from this experiment that invasive alien freshwater species have a limited impact on macroinvertebrate community structure and ecosystem processes when occurring at low densities or densities equivalent to native crayfish, in complex habitats. They alter the abundance of gastropods, primary productivity of periphyton and certain physiochemical attributes such as turbidity and nutrients. However, invasive species (including decapods, Nyström, 2002, Hansen et al., 2013) are typically characterised by achieving high densities in their host ecosystems and this could amplify the impact of the novel traits observed here.

Chapter 5. Ecosystem engineering impacts of native and invasive alien freshwater decapods

5.1 Introduction

Invasive alien species interact with indigenous biota at various trophic levels, causing changes to ecosystems and their services (Simberloff et al., 2012a, Gallardo et al., 2015). Such impacts can occur through the process of ecosystem engineering (Crooks, 2002, Fei et al., 2014) in which organisms alter the physical characteristics of an ecosystem, either through their morphology (autogenic) or other alteration of materials in the environment (allogenic) (Jones et al., 1994). Consequences of altered physical of heterogeneity of an ecosystem at various scales can include changes in: light penetration and general energy flow; flow of oxygen, carbon dioxide or other gases (including mixing); flow of water and other liquids; accumulation of debris and thermal regimes. These physical attributes influence habitat quality for persistence of other organisms, which can feedback to further changes to the physical heterogeneity of the environment and/or the engineer (Jones et al., 2010).

Ecosystem engineering impacts of aquatic decapods include removal of leaf litter, altering the structural heterogeneity of sediment beds (Creed Jr and Reed, 2004). In river basins dominated by riparian plants with slow leaf litter decomposition (mediated by low nutrient, high tannin and lignin content), crayfish can be significant consumers of material that is otherwise unpalatable by other biota, and likely alters the surface structure of the benthos (Schofield et al., 2008). Decapods can also affect sediments: a mesocosm study in France found the invasive alien crayfish *Oronectes limosus* caused erosion of fine sediment (Statzner et al., 2000); similar results of increased fine sediment erosion were found for an endemic New Zealand crayfish

Paranephrops zealandicus in a stream side channel experiment (Usio and Townsend, 2004); and a pond mesocosm experiment in Spain found an increase in suspended solids as a result of the presence of *Procambarus clarkii* (Angeler et al., 2001). Recently in laboratory flume experiments, American Signal Crayfish (*Pacifastacus leniusculus*) have been identified as potential engineers of riverbed topographic change via pit excavation, walking and foraging, leading to increased sediment transport (Johnson et al., 2011, Harvey et al., 2013). This effect has been verified in the field, where sediment transport has been observed in association of increased activity of crayfish (Johnson et al., 2014, Rice et al., 2014).

Changes to the topology of river bed surfaces by crayfish have been accompanied by changes to surface roughness. Changes to surface roughness are important for river basin management because they can affect flow, thus hydrodynamics of river basins (Carling, 1992). Greater bedform roughness can reduce the velocity of flow in rivers, and has implications for flood management (Smith et al., 2014), as well as composition of species specialised to particular flow regimes (Quinn et al., 1996, Hunt and Parry, 1998, Brooks et al., 2005). Aquatic invertebrates have the capacity to increase surface roughness, even at very fine scales (Cardinale et al., 2002). Surface roughness of sediments has been found to be altered by invasive alien crayfish. In flume experiments, *O. limosus* was found to increase surface roughness (Statzner et al., 2000), with similar impacts for *P. leniusculus* in laboratory flume studies (Johnson et al., 2010). In these examples, surface roughness is increased through the creation of mounds from burrowing by crayfish. Direct comparisons of impacts on sediment bed surface roughness between native to invasive alien decapods has so far not been compared.

Geomorphic engineering of river beds by freshwater decapods have rarely been studied in relation to impacts on biodiversity or ecosystem functioning. One exception is the comparisons of flumes containing native *P. zealandicus* crayfish to flumes subjected to artificial sediment disturbance, demonstrating invertebrate taxa vulnerable to habitat change as opposed to crayfish predation (Usio and Townsend, 2004). In the same study, leaf litter decomposition was found to be increased by crayfish, but marginally decreased by sediment erosion in comparison to undisturbed controls. In flume experiments by Statzner et al. (2000, 2002) and Statzner and Sagnes (2008), filamentous algae and biofilm growth was frequently reduced in the presence of bioturbating *O. limosus* crayfish, associated with increased disturbance of sediments. Other than these studies, which lacked comprehensive, multi-trophic appraisals, understanding the impacts of habitat modification by invasive alien decapods on freshwater ecosystems are unclear. Inferences on increased sediment load from decapod bioturbation could be made based on known responses of macroinvertebrates to increases in suspended sediment, especially of fine sediment (associated with sediment transport) (Larsen et al., 2009, Jones et al., 2012, Piggott et al., 2012). Mathers et al. (2016) however found no impacts on sediment-sensitive invertebrate taxa following invasion of *P. leniusculus*, including Ephemeroptera, suggesting the assumed crayfish-derived increase in suspended sediment was not a limiting factor for these taxa. Increases in hydraulic roughness have experimentally also been found to decrease periphyton productivity and invertebrate densities (Quinn et al., 1996), so similar impacts could be expected from decapod-induced increases in sediment surface roughness.

Other engineering effects on biodiversity and ecosystem processes might include altering the structure of sediments through bioturbation (Covich et al., 2004). Bioturbation of sediments by invertebrates can increase the oxygen levels, enabling other species such as aerobic bacteria to access deeper, known as the biological mixing depth (Navel et al., 2012). Biomass in sediments has been found to decrease in the presence of *Procambarus clarkii*, the mechanism attributed to increased mixing of sediment that increases microbial activity (Angeler et al., 2001). Bioturbation also increases the release of nutrients from sediment (Mermillod-Blondin and Rosenberg, 2006), which could amplify nutrient release from decapod excretion (Evans-White and Lamberti, 2006). The extent to which this occurs for other decapod species and for invasive alien species replacing native species is unknown.

Studies on the ecosystem engineering impacts of decapods have been restricted to invasive alien species, and have rarely compared ecosystem engineering of native species of decapod (Harvey et al., 2011). In Europe the native crayfish, *Austropotamobius pallipes*, is being replaced by the invasive alien crayfish *Pacifastacus leniusculus*. Another important invasive decapod is the Chinese Mitten Crab, *Eriocheir sinensis* which has the potential to cause even greater changes to riverbed morphology than *P. leniusculus* due to its intense burrowing activity, large-scale migrations and high localised densities (Lowe et al., 2000, Rudnick et al., 2005).

This study aimed to increase knowledge of any sediment transport, bed morphology and water quality changes following the replacement of native crayfish by invasive alien decapods. Using a field-based flume mesocosm array, the following hypotheses were addressed:

i) Bioturbation by invasive alien decapods would be greater than the native *A. pallipes*, as the invasives are already known to consume more resources than the native (Rosewarne et al 2016) so would be expected to be more active foraging. Impacts would be manifest in greater movement of sediments, with alterations to sediment bed morphology including increased surface roughness through mound creation (*sensu* Johnson et al., 2011).

ii) The increased movement of sediments by invasive alien decapods would cause increase fine sediment transport (cf. Harvey et al., 2013) relative to the native decapods, resulting in higher suspended load.

iii) Macroinvertebrate diversity and community structure will differ for invasive alien decapods compared to natives. Invertebrate species vulnerable to suspended sediment, crayfish predation and changes in hydraulic roughness were predicted to decline in the presence of invasive decapods (Haddaway et al., 2012, Rosewarne et al., 2016);

iv) Decomposition rates would increase through enhanced detritivory by invasive alien species, with sediment respiration increased due to greater bioturbation creating greater biological mixing depths. Biofilm production was expected to decrease through greater disturbance and hydraulic roughness from bioturbation by invasive decapods in relation to the native. Release of nutrients through bioturbation and decapod consumption-excretion was expected to be greater for invasive alien decapods than natives.

5.2 Methods

5.2.1 Experimental set-up

Flume mesocosms in this study were set up to replicate headwater streams with gravel substrates, which are habitats frequented by freshwater decapods (Holdich, 2003). The flumes were located on the University of Leeds Field Research Unit, Spen Farm near Tadcaster, West Yorkshire. Each flume mesocosm consisted of two parallel 3 metre cylindrical PVC channels of 0.3m width, with a header tank, all connected using smaller PVC pipes (Fig 5.1). A ~ 5 cm-deep layer of “natural” gravel (8-0.5 mm size range) was added to each channel, over a ~ 1 cm-deep mixture of sand and bentonite clay (9:1 mixture) to provide a fine sediment source (as used by Harvey et al., 2013). Water from a borehole was added to the header tank and flume, and recirculating flow created with a pump. Water circulated from an inlet pipe to one channel, then the next channel, from which it flowed out of an outlet pipe back to the header tank. To prevent decapod escape, flumes were covered with mesh (20 mm aperture), with the edges fastened down with pegs and duck-tape. To replicate a natural stream ecosystem, channels were seeded with aliquots of detritus, plankton and invertebrates collected by kick sampling a nearby stream (Miller Beck, upstream of Wothersome Lake – latitude: 53.874557, longitude: -1.3947165). This stream has so far has not been invaded by *P. leniusculus* and *A. pallipes* has been observed further downstream below the confluence with Bramham Beck (personal observation on two occasions during baseline kick sampling). Aliquots from the kick sample were made by diluting the kick sample into a 25 gallon tank, homogenizing it by stirring, then adding 1 litre to each mesocosm three times (one litre to each mesocosm first, then the second litre to each mesocosms, etc). These seeding events occurred 1 week before the start of the experiment, then once each week thereafter until the 3rd week to replicate transport of detritus and invertebrates from upstream. Environmental conditions of the mesocosms are given in Table 5.1.

Figure 5.1. Flume mesocosm set up.

Table 5.1. Environmental parameters of the flume mesocosms

Parameter	Mean	St. Dev.
Conductivity (µS cm^{-1})	6.73	1.01
Discharge (m^3 s^{-1})	0.06	0.03
NH_3-N (mg L^{-1})	0.06	0.07
NO_3-N (mg L^{-1})	14.29	2.41
Dissolved oxygen (mg L^{-1})	9.99	0.50
pH	8.22	0.34
PO_4-P (mg L^{-1})	0.00	0.00
Sediment biomass (AFDM g m^{-3})	1.26	0.32
Sediment porosity (%)	56.03	4.68
Water temperature (^oC)	12.1	0.9
Total detritus biomass (AFDM g m^{-2})	3.12	0.26

For the decapod treatments, White Clawed Crayfish were collected by hand from Clapham Beck, Clapham, North Yorkshire (latitude: 54.11, longitude: -2.39). The American Signal Crayfish were collected from Fenay Beck, Huddersfield (latitude: 53.641531, longitude: -1.7309287). Chinese Mitten Crabs were collected from the River Thames at Chiswick Mall (latitude: 51.488489, longitude: -0.24471175). All decapods were held > 1 month in the laboratory to allow acclimatisation, and introduced to the mesocosms following 24 hours of no feeding.

5.2.2 Experimental design

The experiment ran for 28 days (4th October- 2nd November 2014). Treatments consisted of three replicates each of native crayfish (*A. pallipes*), invasive alien crayfish (*P. leniusculus*), invasive alien crab (*E. sinensis*) treatments, and a control with no decapods. These were arranged in a randomised block design. Each mesocosm had two sub-adult decapods (one male, one female, carapace length: 31.53 ± 4.08 mm for White Clawed Crayfish; 34.58 ± 0.28 mm for American Signal Crayfish; carapace width 35.52 ± 4.67 mm for Chinese Mitten Crabs), giving a density of 1.1 m^{-2}, with the exception of the control mesocosms with no decapods added. These densities were generally within the range of those observed in field sites (Nyström, 2002, Rudnick et al., 2003). Decapods of each species were observed to be active at night time in the mesocosms, moving over the sediment and feeding. Microhabitat usage within the mesocosms included in and under the connecting pipes, under leaf packs and under tiles. No burrows were observed other than those excavated under existing structures.

5.2.3 Sediment transport and movement

Suspended sediment concentration was measured at the start and the end of the experiment by taking a 500 ml water sample (at 1500 hrs) and filtering through 0.7 µm GF/F filter discs. These filtered samples had sediment mass measured through drying for 24 hours at 70^{o}C, then the organic fraction estimated by ashing at 500^{o}C (following Ramchunder et al., 2011). The majority of SSC samples were taken throughout dark hours because crayfish are nocturnal, and spikes in suspended sediment are associated with crayfish bioturbation have been observed elsewhere at these times (Harvey et al., 2013, Rice et al., 2014).

Sediment surface topology was measured at the beginning and end of the experiment using structure from motion analysis (Smith and Vericat, 2015). This consisted of drawing down water in the flumes and an array of at least 15 photographs of the flume sediment taken evenly from all angles encompassing the flume mesocosm, at approximately equal height (~1.5 m). Photographs for each flume from the start and end of the experiment were uploaded to Agisoft Photoscan Professional 1.0.4. and pre-measured ground control points to georeference the aligned 3D model. Calculations for the ground control point errors that occur through differences in camera pixels are given in Table 5.2. Digital elevation models (D.E.M.s) were derived from the Agisoft output using the ToPCAT package to a 0.005 m cell size, and the resulting txt. file converted to raster files on ArcMap. Edges of the flumes were removed using the erase feature to focus changes to the sediment and exclude edge effects. To exclude errors and uncertainties arising from subgrid artefacts in the two DEMs, a threshold minimum level of detection (MinLoD) was calculated based on the detrended standard deviations of elevation (Smith and Vericat, 2015). This was incorporated into a conditional raster, which was used to mask the DEM of difference (DoD) so only significant changes were detected. The final DoD was exported into a spreadsheet where net change per cell was calculated as the mean of erosion (negative values) and deposition (positive values), with absolute change per cell calculated as the mean of absolute change (negative values treated as positive). Surface roughness of the final DEM was also extracted using the detrended standard deviation of sediment elevation. To determine directly whether bioturbation had affected pore-spaces in sediment, sediment porosity was measured using the void fraction method from sediment cores (see next sentence for details), which is ratio of the volume of water required to immerse a given volume of sediment (Dullien, 2012).

Table 5.2. Summary of georeferencing errors from ground control points, calculated on Agisoft

	Total error (m)		Error (pix)	
	Before	After	Before	After
Mean errors	0.035	0.188	1.784	9.325
Stdev errors	0.068	0.552	1.670	27.4

5.2.4 Biodiversity and ecosystem functioning

Sediment was collected in cores (0.06 ± 0.01 m^3) before the outlet of each flume (so to sample the section that has received all metabolic products from upstream). These cores were placed in 550 ml chambers which were half filled with water from the mesocosm, and inverted three times to remove air bubbles. Dissolved oxygen in the water column was measured with a YSI Environmental ProODO^{TM} probe and logger, and the chamber kept in absolute darkness for three hours. At the end of this incubation, dissolved oxygen was measured again, and respiration calculated following Doering et al. (2011). The sediment core was then dried at 105^{o}C for 24 hours to measure dry mass, and ignited at 500^{o}C for 2 hours to measure ash free dry mass.

To measure benthic biofilm algal accrual, an unglazed tile was placed in each mesocosm at the start of the experiment and biofilm removed at the end with a nylon brush rinsed with deionised water up to 50 ml. A 5 ml sub-sample of that slurry was first processed for chlorophyll a to measure primary productivity, and the remainder filtered through 0.7 µm GF/F filters, dried at 105^{o}C and ashed at 500^{o}C to measure total biomass of the biofilm following Steinman et al. (1996).

At the end of the experiment, invertebrates were sampled using a modified Surber method where the entire sediment bed was disturbed into a net (250 µm aperture) from the lowest point downstream in the flume upwards. The resulting sample was treated with 70% ethanol before being transferred to the laboratory where macroinvertebrates were sorted and preserved in 70% ethanol. These were identified as close to species-level as possible (using Dobson et al., 2012 and references therein).

Standing stocks of detritus were measured by separating leaves and other woody detritus >10 mm from the rest of the sample over 10mm sieves. Coarse particulate organic matter (CPOM - 10-1mm) was captured on a 1 mm sieve, and fine particulate organic matter (FPOM - 1mm-0.7µm) that passed through the sieve was retained. FPOM was then diluted into 4 litres of deionised water, homogenised and an aliquot of 50ml was filtered through 0.7µm GF/F discs, dried at 50^{o}C and ashed at 500^{o}C to estimate ash free dry biomass. The remainder of the detritus was lightly rinsed, dried and ashed as for FPOM. To estimate leaf litter decomposition rates, four leaf packs of 5g Alder (*Alnus glutinosa*) leaves previously dried at 50^{o}C in 10 mm aperture nylon mesh were added to each mesocosm. These were added at the start, and removed at the end, where they were rinsed gently with deionised water to remove invertebrates, and processed following Benfield (2006).

Water samples were taken at the end of the experiment, filtered through 0.45 µm nylon and processed for dissolved nutrients (ammonia, nitrate, phosphate) using a Skaler SAN ++ continuous flow auto-analyser. Dissolved carbon was also measured from these water samples using a Analytik Jena Multi NC2100 combustion analyser. Temperature and dissolved oxygen were measured over 24 hours at 15 minute intervals using a YSI Environmental ProODO^{TM} probe and data logger in the first and final weeks of the experiment. Electrical conductivity and pH were also measured at the end using an Hanna HI-9835 conductivity meter and a HOBO pH meter, respectively.

All statistics were analysed using R (R v.3.1.0.; R Development Core Team, 2014). To contrast macroinvertebrate community structure between treatments, square root transformed invertebrate abundance data were subjected to ordination analysis using 2D non-metric dimensional scaling (NMDS) based on Bray-Curtis dissimilarities (formula: metaMDS, library: vegan), and using PERMANOVA (formula: adonis, library: vegan) to test for any compositional differences between treatments. Taxonomic richness and Shannon diversity were compared between treatments. Invertebrate taxa that contributed to mean Bray-Curtis dissimilarities between treatments were identified using SIMPER analysis (Clarke and Warwick, 2001) (Supplemental Table 5.1), and between treatment densities (individuals per m^2 of flume) of those taxa were analysed. Densities of known decapod prey (snails, amphipods and chironomid larvae) were also compared across treatments.

Generalised Linear Models (formula: glm) were used to compare treatments against response variables. Distribution of the data family in the glms were specified from maximum likelihood estimates (library: MASS, formula: testmetric, Ripley et al., 2015). Post-hoc Tukey tests (using formula: glht, library: multcomp) were used to test for differences between treatments.

5.3 Results

5.3.1 Sediment transport and movement

DoDs showed a net increase in elevation of the sediment beds in all mesocosms (Fig 5.2). There was no significant change detected in net (Fig 5.3a) and absolute (Fig 5.3b) sediment topological change between either decapod species or decapod-free controls, though the values were generally higher and more variable for all decapod species relative to the controls. Surface roughness, while lower for all decapod species relative to controls, also did not differ significantly (Fig 5.3c).

Suspended sediment concentration did not differ significantly either at the start (day 7) or the end of the experiment (day 27), but was significantly lower for all treatments on day 27 (Wilcoxon test: W= 118, p = 0.004) (Table 5.3, Fig 5.4a). However, suspended organic matter, which did not differ significantly during the first week of the experiment, was much lower in the control treatment and uniformly high across decapod species at the end of the experiment (Table 5.3, Fig 5.4b). None of the nutrients, dissolved oxygen, pH or electrical conductivity measured differed between treatments (Table 5.4, Figs 5.5-5.6).

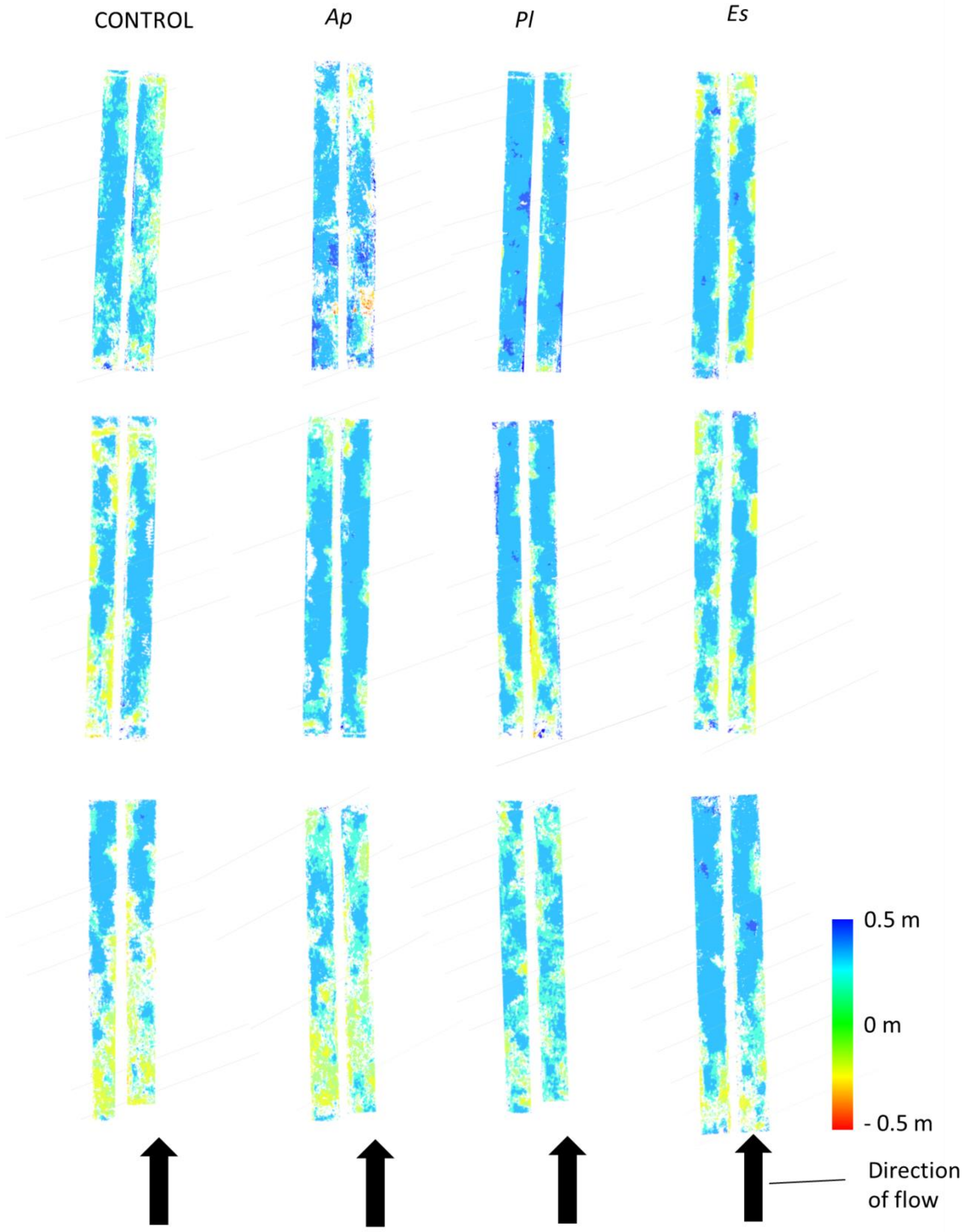

Figure 5.2. Digital elevation models of difference (DoDs) for each treatment (N.B. graphics are arranged by treatment, not by the factorial design).

Table 5.3. Generalised linear model summary statistics for sediment topology and transport parameters

Parameter	**df**	**Residual deviance**	**Pr(>Chi)**
Net change in sediment topology	3	0.006	0.532
Absolute change in sediment topology	3	0.001	0.833
Roughness	3	0.000	0.896
Sediment porosity	3	1.283	0.335
Suspended sediment concentration (10d)	3	2.432	0.869
Suspended sediment concentration (27d)	3	0.899	0.112
Suspended organic matter (10d)	3	2.490	0.436
Suspended organic matter (27d)	3	32.09	**0.030**

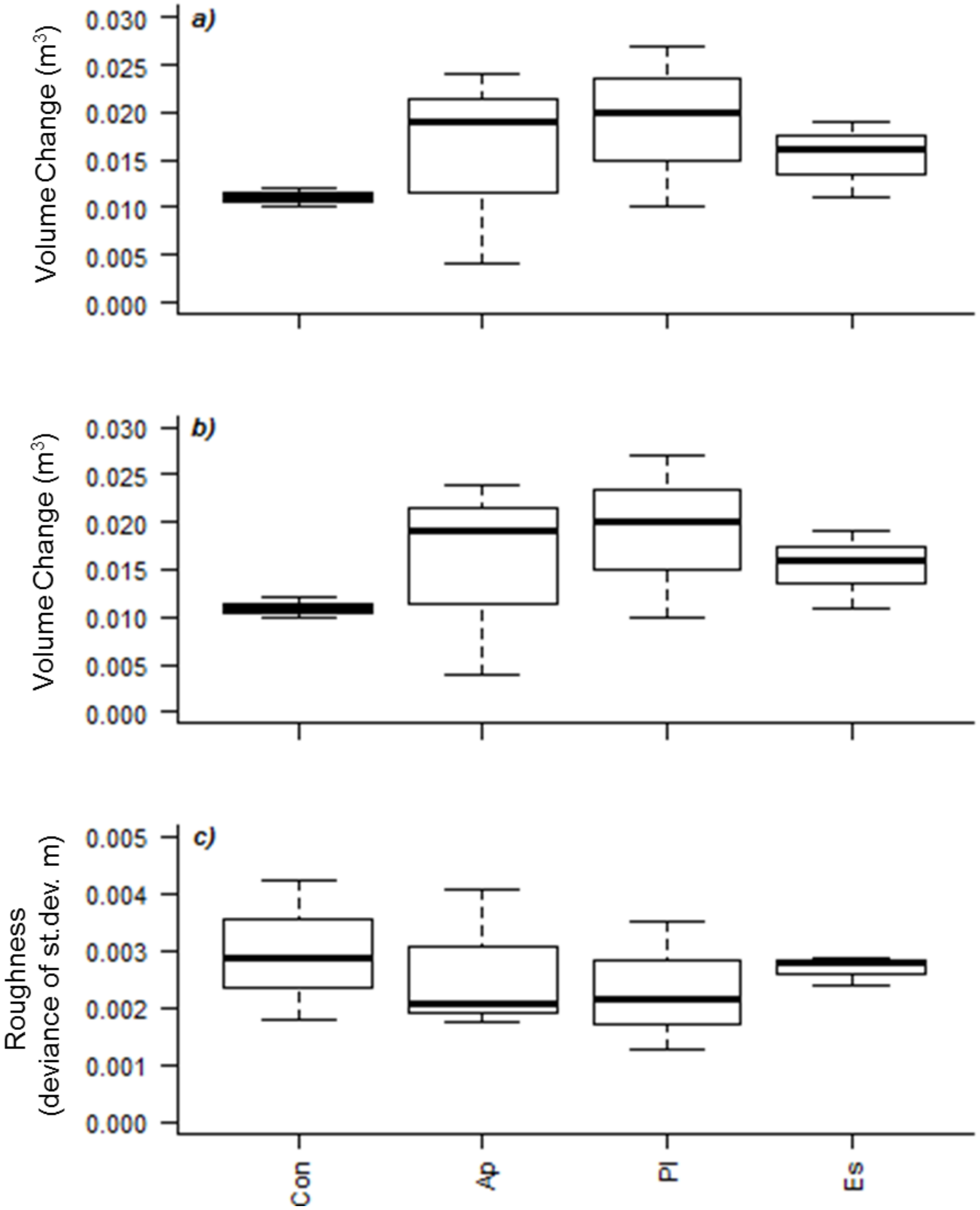

Figure 5.3. Sediment topology variables: a) Net sediment volume change per cell based on DoD models of sediment movement from before and after the experiment. This treats sediment movement in regard to erosion-deposition modelling based on the digital elevation model of difference (DoD); b) mean absolute sediment volume change per cell, treating sediment movement as for net sediment volume change; c) Mean roughness of flume sediment surface as measured by mean deviations of standard deviation of cell sediment elevation, as measured using SfM. Abbreviations for x-axis: Control (Con), *A. pallipes* (*Ap*), *P.leniusculus* (*Pl*) and *E. sinensis* (*Es*).

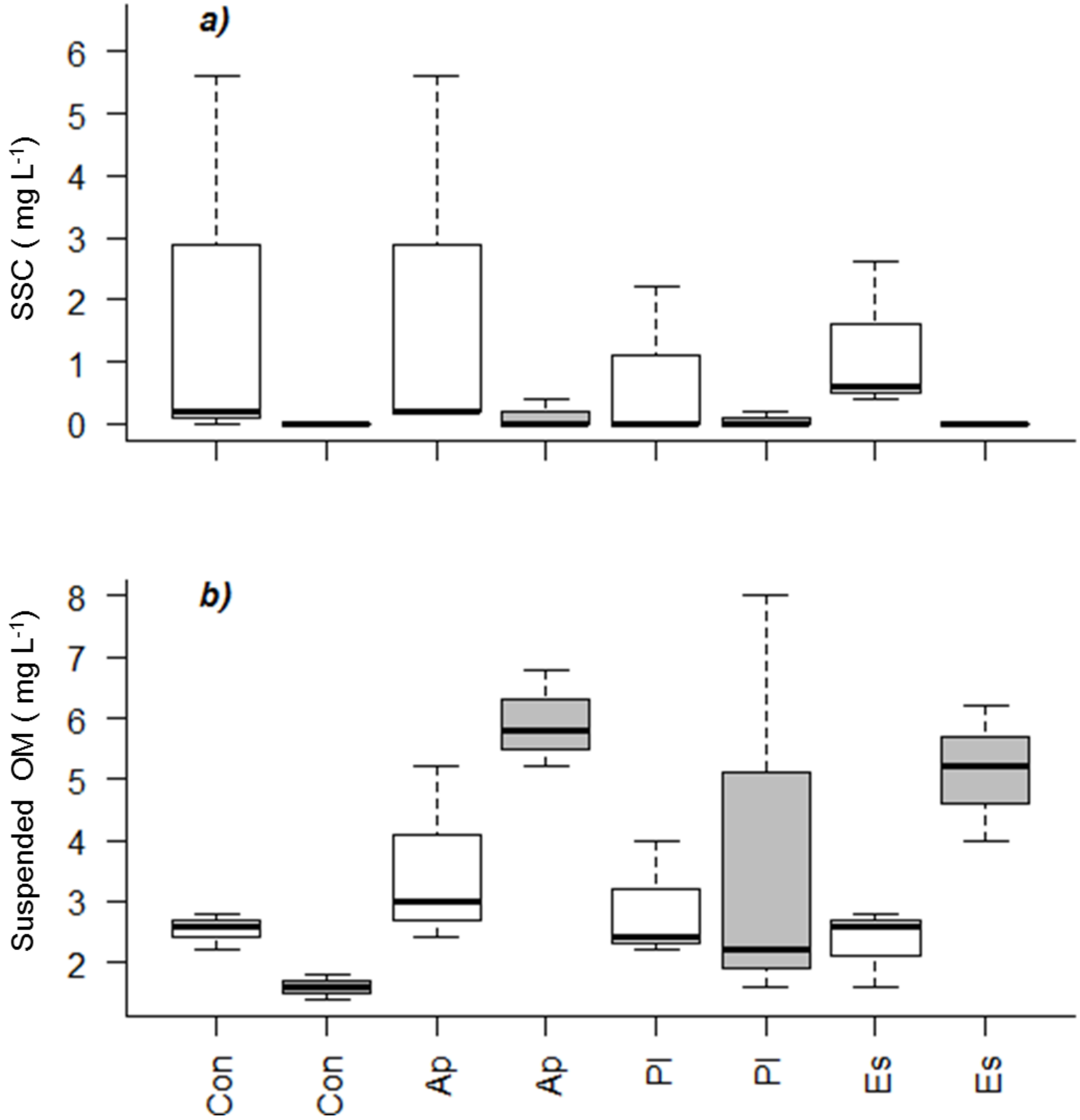

Figure 5.4. Suspended sediment concentration (SSC) (a), and suspended organic matter (OM) (b) at 10 (white bars) and 27 days (grey bars) into the experiment. Abbreviations for x-axis as for Fig 5.3.

Table 5.4. Generalised linear model summary statistics for ecosystem properties and processes

Parameter	**Df**	**Residual deviance**	**Pr(>Chi)**
Decomposition rate	3	0.192	0.395
Detrital standing stock:			
- > 10 mm	3	1.774	0.189
- 10-1 mm (CPOM)	3	0.387	0.656
- 1 mm – 0.7 µm (FPOM)	3	0.032	0.887
Dissolved organic carbon	3	41.16	0.739
Sediment organic matter	3	0.94	0.786
Sediment respiration	3	>0.001	**0.001**
Biofilm biomass accrual	3	>0.001	0.573
Biofilm primary productivity (chlorophyll a)	3	0.062	0.359
Ammonia concentration	3	0.1668	0.378
Nitrate concentration	3	11.31	0.633
Phosphate concentration	3	>0.001	0.214
Dissolved oxygen	3	0.979	0.225
pH	3	0.312	0.453
Electrical Conductivity	3	4.315	0.180

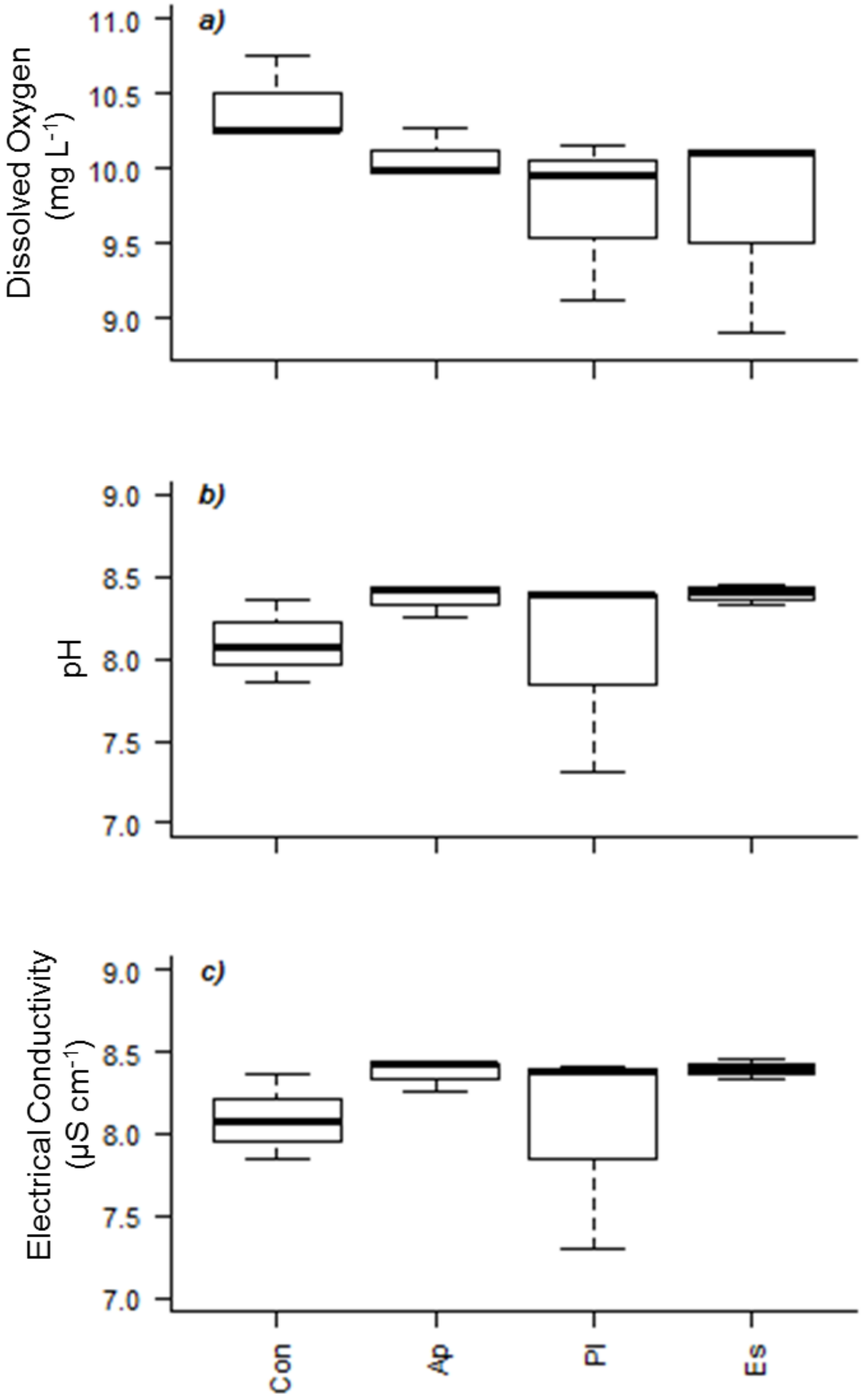

Figure 5.5. Physical water quality parameters: a) mean dissolved oxygen measured over 24 hours; b) pH; c) electrical conductivity.

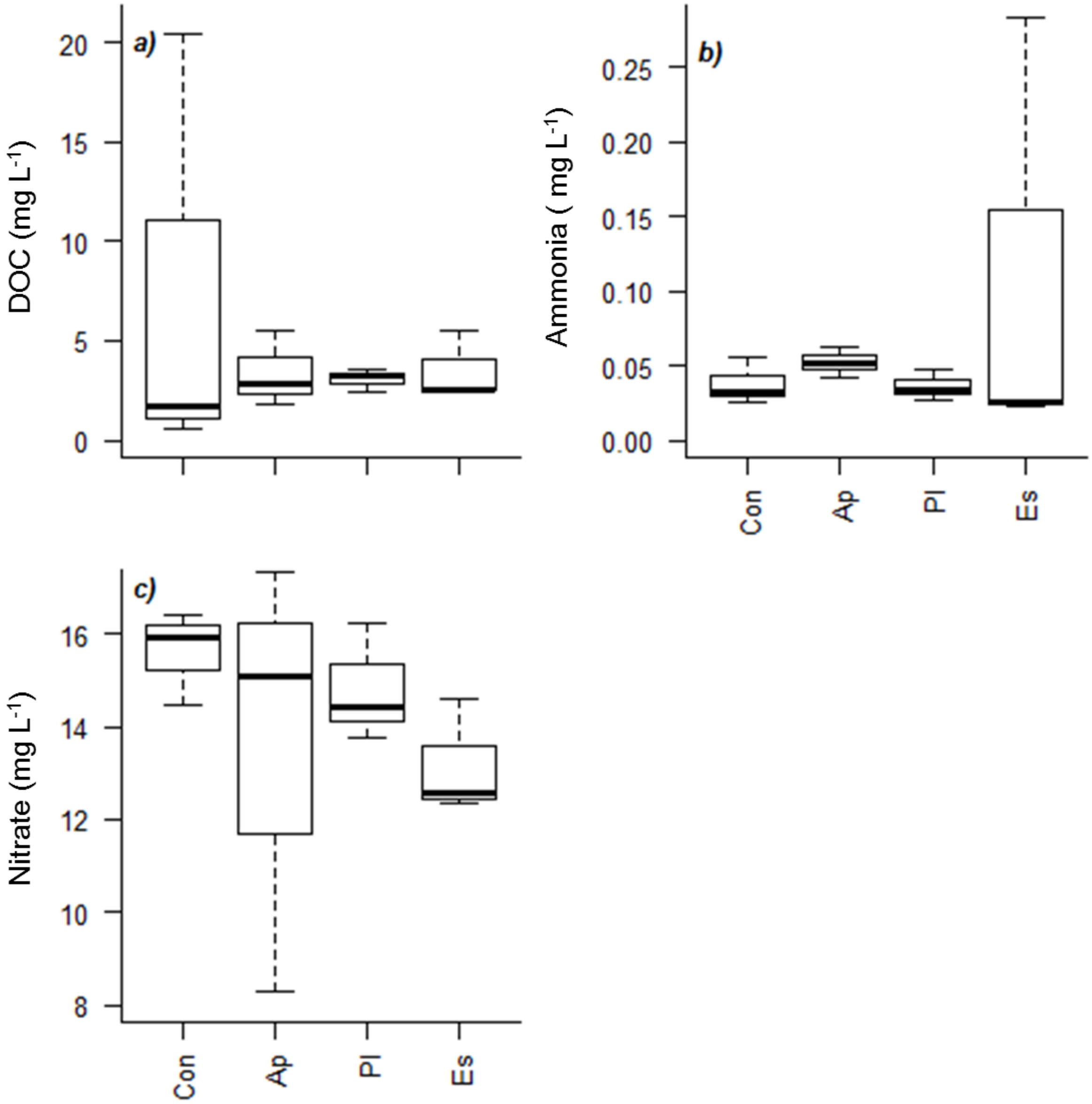

Figure 5.6. Dissolved nutrients: a) dissolved organic carbon, b) ammoniacal nitrogen, c) nitrate. Soluble reactive phosphorous was shown to be negligible and is not shown. Abbreviations for x-axis as for Fig 5.3.

5.3.2 Biodiversity and invertebrate density

Macroinvertebrate community structure differed considerably between treatments (Fig 5.7, Table 5.5), as did taxonomic richness and Shannon diversity, the latter two being higher in both invasive decapod treatments (Fig 5.8a-b, Table 5.6). SIMPER analysis showed that taxa contributing most to dissimilarities were *Baetis rhodani*, Culicidae, *Gammarus pulex*, *Dasyhelea* sp. (Ceratopogonidae), Chironomidae, and the invasive alien snail *Potamopyrgus antipodarum* (Supplemental Table 5.1). There were significantly higher densities of *B. rhodani*, Culicidae and *Dasyhelea* sp. in invasive alien treatments and also (less significantly) more chironomid larvae, and the invasive alien snail *Potamopyrgus antipodarum* (Table 5.6, Fig 5.8c-h). *Gammarus pulex* was abundant in all mesocosms and did not differ between treatments.

Table 5.5. Macroinvertebrate community analysis using PERMANOVA. *p*-values in bold are <0.05.

Term	**Degrees of freedom**	**SS**	**Mean SS**	**F. Model**	**R^2**	**P-value (>F)**
Treatment	3	0.23	0.08	1.74	0.39	**0.03**
Residuals	8	0.35	0.04		0.60	
Total	11	0.59			1.00	

Table 5.6. Generalised linear models summary statistics for the invertebrate metrics

Parameter	df	Residual deviance	Pr(>Chi)
Taxonomic richness	3	86.25	**0.008**
Shannon diversity	3	0.390	**<0.001**
Gammarus pulex density	3	3022.9	0.761
Potamopyrgus antipodarum density	3	4.14	0.774
Baetis rhodani density	3	877.50	**<0.001**
Chironomidae density	3	195001.00	0.512
Ceratopogonidae density	3	49.95	**0.028**
Culicidae density	3	47.15	**0.014**

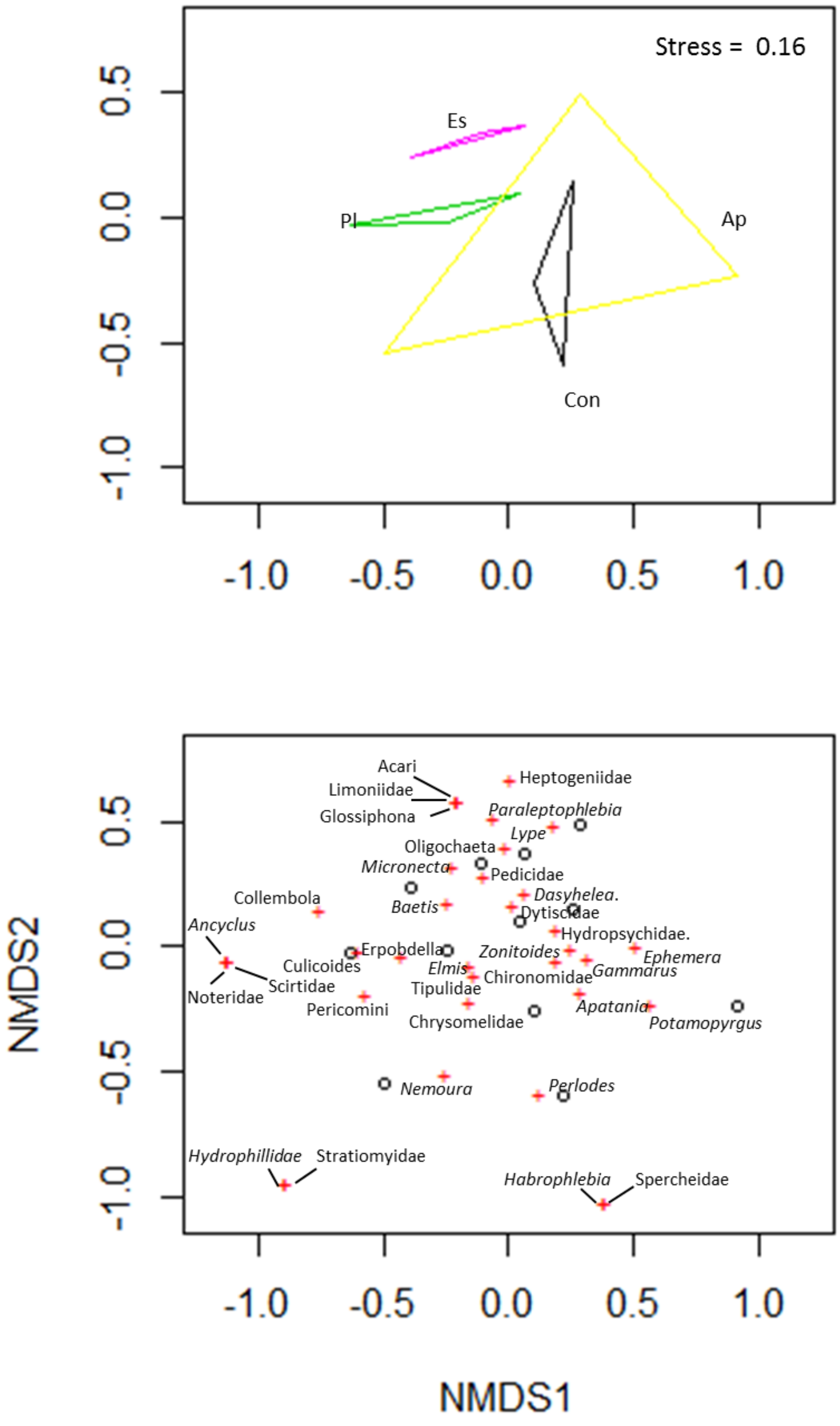

Figure 5.7. Non-metric dimensional scaling of Bray Curtis similarity matrix of square-route transformed macroinvertebrate community. Top panel shows influence of the treatments on community dissimilarities, with the bottom panel showing contribution of each taxon to the ordination. Abbreviations as for Fig 5.3.

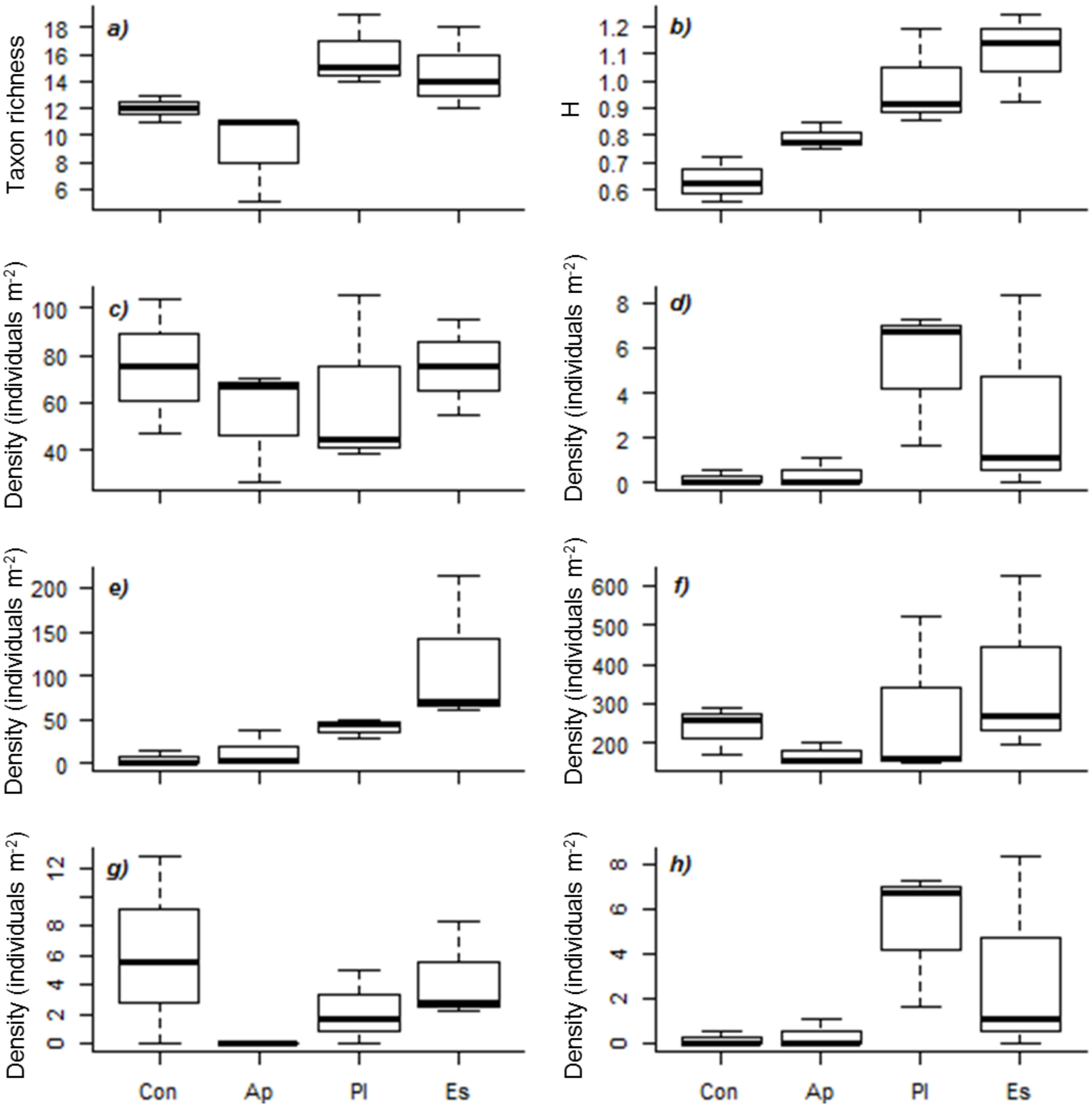

Figure 5.8. Invertebrate parameters of the flume mesocosm experiment: a) taxonomic richness; b) Shannon diversity; c) density of *Gammarus pulex;* d) density of *Potamopyrgus antipodarum*; e) density of *Baetis* sp. larvae; f) density of chironomid larvae; g) density of *Dasyhelea* sp. larvae; h) density of Culicidae larvae. Abbreviations for x-axis as for Fig 5.3.

5.3.3 Ecosystem functioning

Leaf litter decomposition rates, while on average greater for crayfish treatments, also did not differ significantly (Table 5.4, Fig 5.9a). FPOM, CPOM and larger detritus did not differ significantly between treatments, despite larger detritus being substantially reduced in *E. sinensis* treatments (Table 5.4, Fig 5.9b). Sediment respiration was significantly higher for treatments with *P. leniusculus* and controls compared to *A. pallipes* and *E. sinensis* (Fig 5.10a). Organic matter concentration in sediment did not differ significantly across treatments (Fig 5.10b). Biofilm accrual and primary productivity were not significantly different between treatments (Table 5.4, Figs 10c-d).

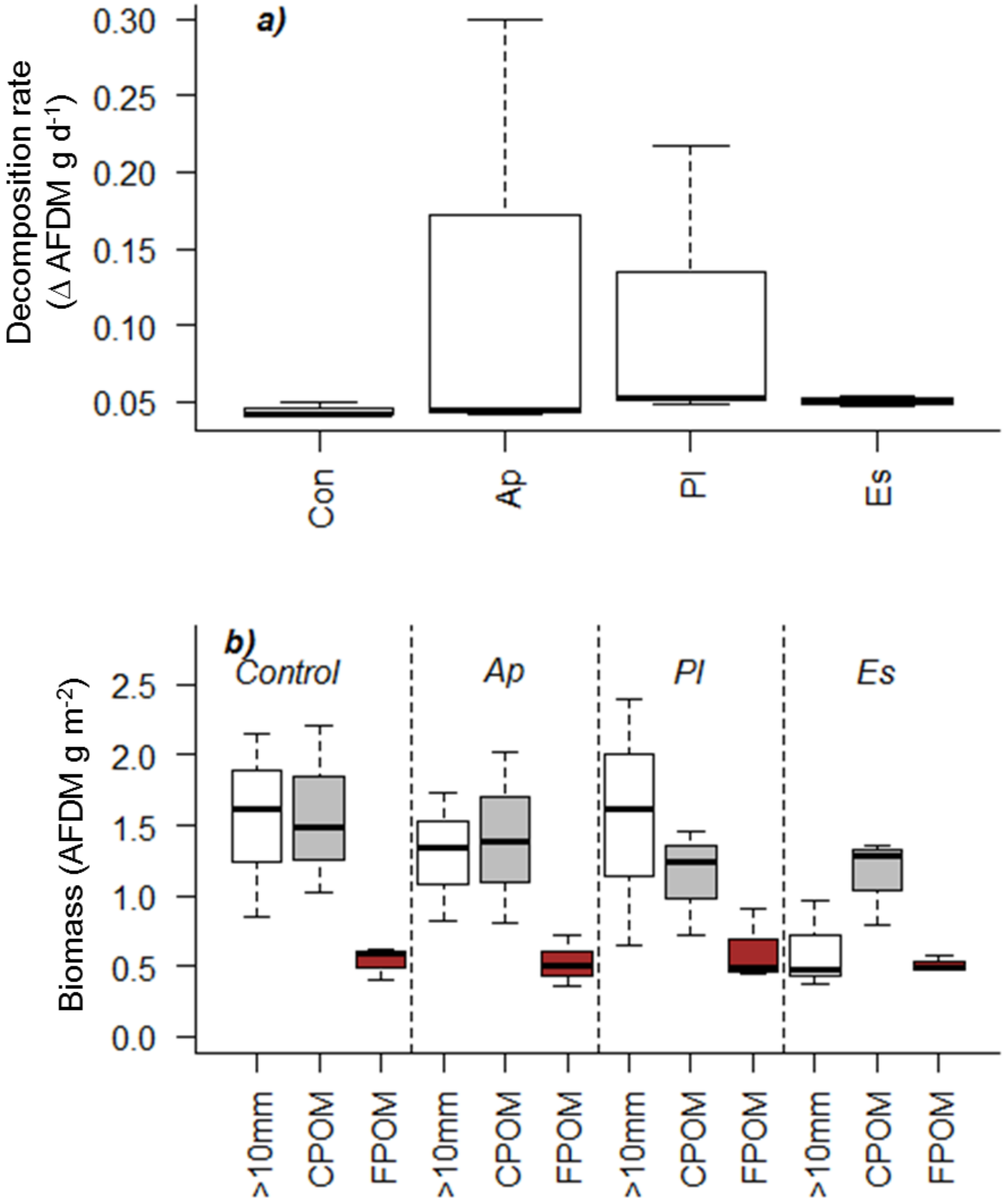

Figure 5.9. Leaf pack decomposition rates (a) and standing stocks (b) from the flume experiment. For the standing stocks, detitrus size is divided by matter > 10 mm (white bars), CPOM <10>1mm (grey bars) and FPOM <1mm>0.7 µ (brown bars), for Control (Con), *A. pallipes* (*Ap*), *P.leniusculus* (*Pl*) and *E. sinensis* (*Es*) treatments.

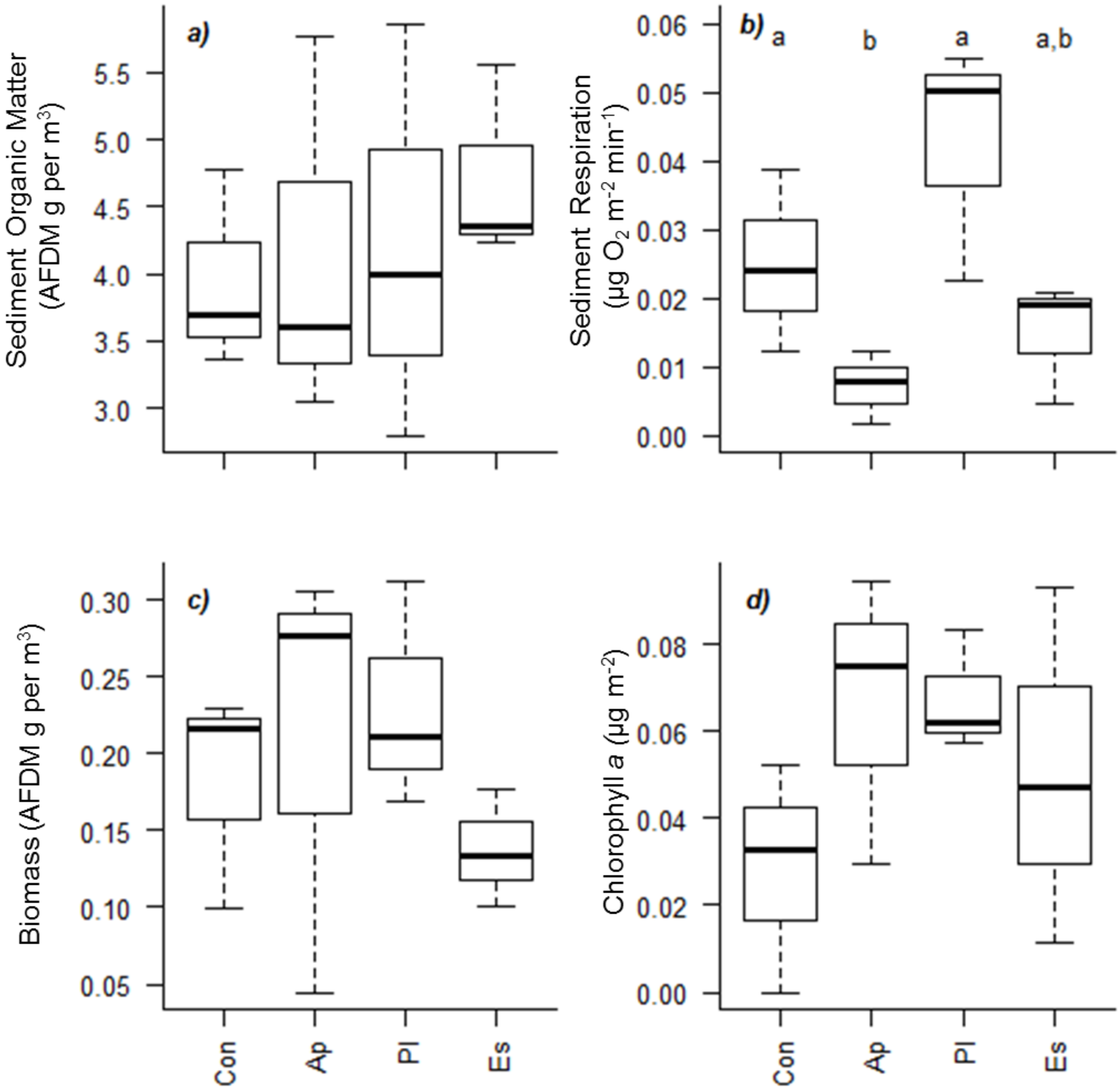

Figure 5.10. Benthic productivity and respiration: (a) Sediment organic matter biomass; (b) sediment respiration with letters denoting groups based on *post hoc* tests; (c) biomass accrual of biofilms; and (d) primary productivity of periphyton.

5.4 Discussion

This study is the first direct comparison of the impacts on benthic ecosystem engineering, biodiversity and ecosystem functioning by the invasive alien decapods *P. leniusculus* and *E. sinensis* in comparison to the native *A. pallipes*. Sediment movement and morphology was altered by all species similarly, but not significantly after the 27 days. Sediment transport *per se* was also not significantly affected by decapod species, but suspended organic matter transport was significantly higher for decapods compared to controls but not between each other. Impacts on biota and ecosystem functioning were restricted to certain invertebrates and sediment respiration, and are attributed to the observed increase in suspended of organic matter.

4.4.1 Sediment movement and transport

Sediment movement was not observed to be greater in the presence of decapods. This was not expected so H_1 is rejected; this could have been explained by the presence of hides such as the u-bend in the flume, leaf packs and beneath tiles (all decapods were found in these microhabitats). These hides meant that the decapods did not need to excavate burrows in the unconsolidated benthic sediments. Previous studies have examined bioturbatory effects of crayfish in unrealistically homogenous sediment beds in the laboratory, and in the absence of objects that could have acted as hides (Johnson et al., 2010, Johnson et al., 2011). This is supported by one experiment showing an increase in sediment transport in the presence of crayfish following removal of refugia (Statzner et al., 2000). Results observed in the present study were thus likely to be derived from engineering traits of decapods feeding, moving over sediment and intra-specific interactions (Harvey et al., 2011). Similarly, surface roughness, did not differ between treatments, unlike published studies showing increased roughness in the presence of crayfish in aquaria and flume mesocosms through creation of pits and mounds (Statzner et al., 2000, Johnson et al., 2010). Decapod species could therefore have minimal impacts on sediments if river beds would be structurally heterogeneous with numerous natural refugia such as boulders, tree routes, fallen logs, etc. Further study using this mesocosm system without refugia present would help test this hypothesis, which would have bearing on managing invasive alien decapods that are shown to impact sediment beds *in situ* (Harvey et al., 2013, Rice et al., 2014).

Contrary to predictions (H_2), sediment was not found to have been moved significantly, however, there were some changes to fine sediment transport likely caused by decapod bioturbation. SSC declined over time for all treatments. This most likely indicates that both decapods and other invertebrates had settled in the mesocosms following establishing stable refuges, resulting in smaller bioturbation effects. Suspended organic matter increased for decapod treatments, but this is likely a result of increased shredding of organic matter by decapods, and entrainment of small fragments than bioturbation *per se*.

The results of this study suggest that it might not be possible to linearly extrapolate results of simplified laboratory experiments on decapod ecosystem engineering (e.g. Johnson et al., 2010, 2011, Harvey et al 2013) to all real-world river ecosystems. Similarly, the heterogeneity of river beds with regard to debris that could be used for shelter (such as logs or large rocks) in field studies on crayfish and crabs has usually not been reported, as the focus has predominantly been on burrows in soft, fine sediments (Rudnick et al., 2005, Harvey et al., 2013). Contexts affecting invasive alien species engineering impacts on sediment should now be considered for impact assessments, particularly the type of sediment (coarse, immovable versus fine, easily disturbed sediments) in different types of aquatic habitat.

4.4.2 Biodiversity and ecosystem functioning responses

A significant finding from this experiment was that the macroinvertebrate communities in the flume mesocosms were structurally different in both the invasive decapod treatments, offering support for H_3. This included increased taxonomic richness and diversity indices, with increased densities of taxa such as *Baetis rhodani*, Culicidae, and Ceratopogonidae. Prey species of the decapods, notably *Gammarus pulex* showed no difference in density between treatments. This was unexpected considering both laboratory and mesocosm studies have observed large effects of *A. pallipes*, and to a greater extent, *P. leniusculus* and *E. sinensis* on *G. pulex* (Haddaway et al., 2012, Rosewarne et al., 2013, Rosewarne et al., 2016) but is consistent with some field studies (Mathers et al., 2016). As with the engineering effects, this highlights the difficulty of predicting outcomes of ecological effects in complex systems based on simplified laboratory experiments. The only gastropod species that colonised successfully across treatments (the invasive alien New Zealand Mud Snail, *P. antipodarum*) did not differ significantly in density between treatments, despite snails consistently being depleted in most studies on decapods (Twardochleb et al., 2013), but was still consistent with observations of Mathers et al. (2016). Invertebrate taxa that showed increased density in the presence of invasive alien decapods were collector-gatherers and suspension feeders, and could be responding to re-suspension of organic matter by decapods.

Decomposition of leaf litter and standing stocks of detritus were not different between treatments, despite a predicted increase in decomposition rates for invasive alien decapod treatments (H_4). Both *P. leniusculus* and *E. sinensis* shred leaf litter in the laboratory (Chapter 2 & 3), and also in other mesocosm (Zhang et al., 2004, Rudnick and Resh, 2005) and cage experiments (Lagrue et al., 2014). However, this absence of response has also been observed in mesocosms at this site (Rosewarne et al 2016, Chapter 4), and is hypothesised to be caused by a greater availability of more nutritious prey (Chapter 4). Other leaf shredding invertebrates, notably *G. pulex*, were also unaffected by treatments, and this could have explained the lack of effect too. Detrital processing has therefore been shown to be negligible in both lentic and lotic mesocosms for these species, and potentially highlights a limitation of the prediction value of simplified laboratory experiments for understanding ecological processes in complex real-world ecosystems.

Sediment respiration was higher for *P. leniusculus* compared to *A. pallipes*, but not to controls or *E. sinensis*. This was predicted to occur in H_4, but via bioturbation, for which there was no evidence based on the results from sediment measurements above. Increased sediment respiration might have been explained by greater availability of nutrients (Morris and Bradley, 1999), though *P. leniusculus* appears to retain nutrients in its body rather than excrete them (Usio et al. 2006, Chapter 2), so increased availability of suspended organic matter could have been a factor. This was not accompanied by any differences in organic matter content of the sediment, unlike that observed in ponds for *P. clarkii* by Angeler et al. (2001) or for *P. leniusculus* by (Nyström et al., 1996). In the former study, crayfish occurred at densities of 3.8 individuals m^{-2} and the experiment ran for 18 days. The study by Nyström et al (1996) in comparison was the result of crayfish occurring in ponds for ~ 9 years, for which crayfish abundance varied, being negatively correlated with sediment organic matter content. In contrast, the present study had a density of 1.1 individuals m^{-2} for a period of 30 days. It is therefore possible that sediment organic matter could have differed had the experiment occurred for a longer period of time with greater densities of decapods. These results indicate that *P. leniusculus* has the potential to increase the overall community respiration of rivers in comparison to *A. pallipes*, which in turn could increase the consumption rates of oxygen, and increase the carbon release from invaded rivers (Yvon-Durocher et al., 2010). Conversely, *A. pallipes* and (in part) *E. sinensis* both appear to suppress sediment respiration, but the causes are not clear considering both species have greater nutrient excretion rates compares to *P. leniusculus* (Chapter 2).

This study noted no difference in algal biofilms, in contrast to a flume experiment on *Oronectes limosus* that showed a decrease in filamentous algae and biofilms growing on sediment, which was attributed to crayfish disturbing algae directly and smothering with sediment (Statzner et al., 2000). There was however no evidence of increased sediment build-up on tiles in this study. Decapod-biofilm dynamics in other studies have been in lentic ecosystems, and have been typified by trophic cascades of increased biofilm productivity caused by increased predation upon grazing invertebrates by decapods (Twardochleb et al., 2013, James et al., 2015), though this has now been found to be influenced by turbidity (Chapter 4). The most common biofilm grazing species was the snail *P. antipodarum*, which did not occur at high densities. Other scrapers of periphyton, such as larvae of Heptageniidae were rare, and it is likely no effect was observed due to a low numbers of regulators of biofilm for decapods to impact. Thus, despite community structure being differentiated by invasive alien decapods, it was apparently decoupled from ecosystem functioning variables, and could be attributed to the high representation of collector-gathering taxa.

4.4.3 Conclusions

Both native and invasive alien decapod species had negligible impacts on sediment movement and topology when held at equal densities in controlled flume mesocosms. This is contrary to expectations that invasive alien species are known to affect sediments, however this is likely to have been mediated by the presence of refugia. *A. pallipes* had similar impacts on sediment topology and transport relative to other decapods of equal density and size. As this endangered species (Füreder, 2010) is being translocated into habitats without crayfish (in accordance with IUCN guidelines) (Kozák et al., 2011), it is suggested that impact assessment plans should take this effect into account as there may be wider impacts on other native plants and animals.

Invertebrate community structure was however affected by invasive alien decapods, with collector-gathering species found to be elevated. This is possibly a result of increases suspended organic particulate matter, which might have been a factor for the increased sediment respiration. However, no other ecosystem processes or properties differed as a result of this differential invertebrate community structure. Real world impacts of invasive alien decapods on sediments might differ to the native crayfish however, as they can attain greater densities and larger average body size. These factors are likely to amplify the already observed between-species impacts on the invertebrate assemblage and sediment microbial processes. The effects of this contrasting density and body size on sediment dynamics should now be verified between these invasive decapods and their native analogue so that any negative consequences can be prevented or managed.

Chapter 6. The consequences of losses and gains of freshwater decapod species: a critical evaluation of research techniques, and implications for future research and management

6.1. Introduction

Establishing the impacts of a putative invasive alien species is necessary for the prioritization of management, which could include prevention of spread, eradication, control or no-management (Lodge et al., 1998, Jeschke et al., 2014). Understanding ecological impacts of a biological invasion requires an ability to assess densities achieved, the rate of range expansion and the actual effect per individual or unit biomass of the invading organism (Parker et al., 1999). The latter is a particularly important factor for assessing impacts because, even at low densities, an alien species expanding its range could invoke keystone impacts relative to its abundance (Letnic et al., 2009). Measuring these *per capita* effects will often require microcosm studies, with the 'real-world' outcome verified in more complex experimental venues, such as field-based mesocosms, cage enclosures/exclosures in the field or natural experiments where comparisons are made between invaded and uninvaded localities (Strayer, 2012).

In this thesis, the approaches of microcosms and field based mesocosms are used in order to explore the impact of invasive alien decapods in tightly controlled lab conditions and to scale up to more realistic field settings. Microcosm studies (usually in the laboratory) involve isolating putative invasive alien species and exposing them to an ecosystem attribute of interest, usually a certain resource or microhabitat, and then measuring one or only a few selected impacts (Srivastava et al., 2004, Benton et al., 2007). Advantages of laboratory studies include the opportunity for sufficient replication (hence high statistical power) and the identification of specific ecophysiological responses (such as excretion rates). Disadvantages of microcosms are their lack of realism owing to small size, habitat simplicity or inability to measure ecosystem-scale variables such as multi-species interactions or whole ecosystem processes (Schindler, 1998, Stewart et al., 2013). Mesocosms have the advantage of being larger, enabling greater complexity (such as more species and trophic elements), and if outdoors, capturing natural fluctuations in daylight and temperature (Stewart et al., 2013). Mesocosms can also be subjected to a greater range of ecosystem-level measurements (Harris et al., 2007, Ledger et al., 2009, Brown et al., 2011). In the context of invasive alien species, mesocosms provide advantages in that they can be isolated from natural waterbodies to prevent escape of the focal species, and to create replicated ecosystems that are naïve to the invader. The latter is difficult in cage enclosure/exclosure experiments in natural waterbodies due to possible confounding effects that have been termed "the ghost of invasion past" (Kueffer et al., 2013), where invasive alien species can alter ecosystem states that remain altered even after eradication (Wallem et al., 2010). Ethically, experiments in natural systems can only be deployed responsibly in already invaded ecosystems so not to spread invasive alien species to new ecosystems. However, a disadvantage of both mesocosm and cage experiments is the logistical difficulty and cost of achieving statistically powerful replication (Lodge et al., 1998). *In situ* studies are most powerful when they come in the form of natural experiments (invaded versus uninvaded sites), yet while extremely realistic they often suffer from low replicability, with randomization of sites typically not possible and studies thus being susceptible to confounding factors (Schindler, 1998). While careful stratification of sample sites might be feasible for some invasions, identifying sample sites could be confounded by an absence of available data on the presence/absence and dominance/rarity of the invader.

This thesis investigated the ecological impacts of invasive alien decapod crustaceans replacing a native crayfish species that was previously widespread across Western Europe. Predicting non-redundancy of invasive alien decapods based on published laboratory measurements of predatory functional responses, factorial experiments ranged from studies on biodiversity-ecosystem impacts in laboratory studies (Chapters 2 & 3) and field-based mesocosms (Chapters 4 & 5). Laboratory studies showed clear non-redundancy of invasive alien decapods, but this effect was less clear when experiments were scaled up to larger, more complex experimental venues. For example, leaf litter decomposition rates differed between species strongly in the laboratory, but not in field-based mesocosms. Periphyton productivity did not vary in the laboratory (Chapter 2) or flumes (Chapter 5) but did significantly differ between treatments in the pond mesocosms (Chapter 4).

In this final chapter, the role of scale and complexity in experimental venues used for assessing the effects of freshwater invasions are synthesised and evaluated critically. The experimental venue type, size, and duration were analysed against the ecosystem processes and properties that were measured consistently across the thesis. It was hypothesised that differences in the outcome of invasive alien decapods between laboratory and mesocosm experiments would be caused by the size and complexity of the experimental venue, and possibly also by duration of the experiment. Following a brief review of the applicability of the experiments to the overall research questions of the thesis, the ecosystem-scale consequences of replacement of *A. pallipes* by *P. leniusculus* and *E. sinensis* are discussed. The general research questions were:

(i) Does the type of technique adopted for invasive alien species experiments affect the outcome of impact assessments?

(ii) What are the river basin-scale impacts of invasion of *P. leniusculus* and *E. sinensis* following the extirpation of *A. pallipes*?

6.2 The role of experimental technique for assessing biological invasion effects

6.2.1 Analysis

To measure the effect of experimental technique on the outcome of a biological invasion impact study, the variables measured across all the experiments (laboratory microcosm, water tank [pond] mesocosm, flume mesocosm) in this thesis were combined and standardised where necessary. This included ecosystem functioning variables such as litter decomposition rate and primary productivity; and water quality parameters such as nutrients and pH. Because effects on macroinvertebrates were not studied in the laboratory for this thesis (outcomes already studied by Haddaway et al., 2012, Rosewarne et al., 2016, Taylor & Dunn, unpublished), they are not compared in this study. The properties of the different experimental venues are characterised in Table 6.1.

Generalized linear models were used to differentiate the impact of venue on freshwater ecosystem variables and how it interacts with treatment. To differentiate causality *post hoc* of regulation of decapod-ecosystem responses by experimental venue, GLMs were used with the commonly measured ecosystem variables (decomposition, FPOM, etc) as a response with treatment as a factor with experimental venue type, size (m^2), volume (m^3), area:volume, duration (days) and number of trophic levels as covariates. Mean water temperature of each venue differed because laboratory microcosms were incubated in a cold room, whereas the outdoor mesocosms had more variable thermal regimes driven by meteorological and diurnal fluctuations.

Table 6.1. Overview of ecosystem response variable measured across experimental venues in this thesis

Experimental venue	Type	Sample size (n)	Benthic Surface Area (m^2)	Volume (m^3)	Area: Volume	Duration of experiment (days)	Number of trophic elements
Laboratory	Microcosm	24	0.054	0.0054	10	14	3 (L,B,D)
Water tank (pond)	Lentic mesocosm	4	0.78	0.51	1.52	30	7 (L,P,B, C,Pr,D)
Flume	Lotic mesocosm	3	1.8	0.54	3.3	33	5 (L,B,C,Pr,D)

* abbreviations for trophic levels: L – leaf litter & other detritus; B – microbial biofilms, including bacteria, fungus, diatoms; P – primary producers that include phytoplankton and macrophytes; C – invertebrate primary consumers such as snails, detritivores; Pr – predators (primarily invertebrate predators); D – decapods (which could be assigned as omnivores).

6.2.2 Impact of experimental venue on invasive species-ecosystem relationship

Experimental venue type, area, volume, area:volume, duration and number of trophic levels had significant interactions with decapod species effects on leaf litter decomposition (Table 6.2). However, these interactions were not significant for the other ecosystem functions (Table 6.2). Similarly, none of the water quality variables were associated with a significant interaction between experimental venue, area of the experimental venue or duration of the experiment (Table 6.3). Differences in decomposition, FPOM and sediment respiration were significantly different between the experimental venues, with higher values in the laboratory compared to outdoor mesocosms (Fig 6.1). For dissolved nutrients, nitrates and phosphates were increased and reduced respectively in mesocosms compared to the laboratory (Fig 6.2). pH was particularly elevated for the pond mesocosms relative to both the laboratory and the flume mesocosms. The pH differed between the different mesocosms (water tanks, Chapter 4); flumes (pH mean=8.22, max=8.45, Chapter 5), although pH did not vary between treatments within each study. These high and fluctuating pH values in the water tanks (mean= 9.82, max = 10.24) suggest either high calcareous rock at the borehole or lime deposition in the catchment of the borehole, which is part of a working arable farm. Other variables showed negligible differences between experimental venues, including for biofilm accrual and primary productivity, as well as ammonia (Figs 6.1, 6.2).

Surface area of the experimental venue was the factor providing the best models for FPOM and nitrate, while the interaction between decapod species and trophic level provided the best model for pH and ammonia (Table 6.2). Decapod species provided the best fit for primary productivity of biofilms, which was otherwise not significant across treatments. Other ecosystem response variables had multiple factors of equal AIC values, such as decomposition rate showing equal model fit for decapod*venue and decapod*volume interactions.

6.2.3 Implications for assessing impacts of invasive alien species

This research has provided new comparative evidence for the impacts of invasive alien species on ecosystem responses spanning research venues, from the laboratory to mesocosms. Ecosystem responses measured have included both abiotic and biotic factors, plus their interaction which is novel because most studies of invasive species do not consider these simultaneously. Many studies use laboratory, mesocosm and field methods simultaneously (e.g. Rosewarne et al 2016), though few consider the impact of scaling. Lodge et al. (1998) found consistent impacts of invasive alien crayfish (*Oronectes rusticus*) on snails in the laboratory, in cage experiments and in lakes with different densities of crayfish. Similarly, another study examining impacts of invasive freshwater fish (*Pseudorasbora parva*) on trophic niche divergence found consistent results across pond mesocosms, small *in-situ* ponds and large *in-situ* ponds (Tran et al., 2015). In parallels to grazer-algae interactions, a meta-analysis found longer study duration, greater variance and less replication in field compared to laboratory studies, as with this chapter, but that these factors only explained a small proportion of the variation of the meta-analysis (Hillebrand and Gurevitch, 2014). The results of this chapter show the contrary of these studies: impacts of invasive alien decapods were typically incongruent across scales. Factors predicting responses instead included the area of the experimental venue, and the interaction between decapod species and number of trophic levels.

The finding of incongruence is an example of an emerging theme in ecology, context (O'Connor and Donohue, 2013), where different sizes, time scales and trophic levels differed across the experiments. This *post-hoc* review of the inferential impact of scale on ecological impacts of invasive alien species is limited by the differing contexts of each of the experiments. This includes co-factors not necessarily linked to scale, such as substrate or occurrence of other species, which could induce feedbacks that could affect other regulatory factors in the mesocosm. In this study, trophic elements varied by venue, and while this was more logistically-driven than by design, trophic element diversity is positively associated with scale in outdoor experiments and surveys (Post et al., 2000). In this thesis, the occurrence of alternate food sources was hypothesised to have caused the reduced decomposition rates in the more taxonomically rich mesocosms (Chapter 4 & 5). Presence of shredding invertebrates that degrade leaf litter differently, as well as become consumed at different rates by decapods might have also caused neutral impacts – if an ecosystem holds resources more nutritious than leaf litter, then an invasive decapod would be predicted to consume those preferentially (Magoulick and Piercey, 2016).

To prospectively test the impacts of scale on ecosystem responses to invasive alien decapods (and other species), mesocosms of different sizes (area and volume) could be designed, with trophic levels, resource density and physicochemical properties such as thermal regime controlled. These responses could then be applied using varied contexts, such as different substrates, benthic invertebrates or other conditions or resources. The realism of experiments has been presented as a challenge to predicting the impacts of extinction of *A. pallipes* and its replacement by *P. leniusculus* and *E. sinensis*. Certain elements of realism were necessarily excluded to enable consistent comparisons, such as excluding avian predators of decapod (*i.e.* Heron, *Ardea cinerea*). Fish and amphibians were also not included in the mesocosms primarily due to their uncertain suitability to hold such animals, legislative-logistical issues and the potential for amphibians to move between mesocosms.

While this chapter highlights the need for caution when scaling impacts of invasive alien species from the laboratory to the field, microcosms are still useful in ecology for formulating predictions to understand full scale ecosystems (Benton et al., 2007, Brown et al., 2011). In invasion biology, microcosm experiments examining individual species can identify traits that could signify impactfulness (Dick et al., 2014). For example, the predatory functional responses of *P. leniusculus* and *E. sinensis* measured in the laboratory have concurred with observations in mesocosm experiments and *in situ* (Haddaway et al., 2012, Rosewarne et al., 2016, Mathers et al., 2016).

Table 6.2. Generalized linear models assessing interactions between factorial decapod treatments and experimental venue for various ecosystem functioning variables

Response variable	**Decomposition (Δ AFDM g d^{-1})**	**FPOM (AFDM g d^{-1})**	**Biofilm biomass (AFDM g m^{-2})**	**Biofilm Primary productivity (chlorophyll *a* µg L^{-1})**
Factor [df] ↓				
Decapod species [3]	d: 0.067 **Pr: <0.001** AIC: -450	d: 0.226 Pr: 0.4811 AIC: 59	d:0.000 Pr: 0.476 AIC: -1386	d: 0.194 Pr: 0.622 AIC: 82
Experimental venue type [1]	d:0.018 **Pr:0.002** AIC:-424	d: 3.483 **Pr: <0.001** AIC: 11	d: 0.000 Pr: 0.219 AIC: -1389	d: 0.032 Pr: 0.584 AIC: 80
Venue area [1]	d:0.011 **Pr: 0.014** AIC: -420	d: 7.767 **Pr: <0.001** AIC: -92	d: 0.000 Pr: 0.277 AIC: -1389	d: 0.055 Pr: 0.479 AIC: 80
Venue volume [1]	d: 0.018 **Pr: 0.002** AIC: -424	d: 3.796 **Pr: <0.001** AIC: 6	d: 0.000 Pr: 0.219 AIC: -1389	d: ,0.035 Pr: 0.573 AIC: 80
Area:volume [1]	d: 0.018 **Pr: 0.001** AIC: -424	d: 2.316 **Pr: <0.001** AIC: 29	d: 0.000 Pr: 0.221 AIC: -1389	d: 0.025 Pr: 0.631 AIC: 80
Duration of experiment [1]	d: 0.018 **Pr: 0.001** AIC: -424	d: 2.61 **Pr: <0.001** AIC: 25	d: 0.000 Pr: 0.220 AIC: -1389	d: 0.027 Pr: 0.618 AIC: 80
No. trophic elements [1]	d: 0.018 **Pr: 0.002** AIC: -424	d: 0.789 **Pr: 0.002** AIC: 49	d: 0.000 Pr: 0.243 AIC: -1389	d: 0.013 Pr: 0.726 AIC: 80
Decapod*venue type [3]	d: 0.03 **Pr: <0.001** AIC: -486	d: 0.061 Pr: 0.812 AIC: 21	d: 0.000 Pr: 0.869 AIC: -1381	d: 0.019 Pr: 0.982 AIC: 90
Decapod*venue area [3]	d: 0.03 **Pr: <0.001** AIC:-479	d: 0.038 Pr: 0.698 AIC: -85	d: 0.000 Pr: 0.890 AIC: -1381	d: 0.024 Pr: 0.975 AIC: 90
Decapod*venue volume [3]	d: 0.03 **Pr: <0.001** AIC: -486	d: 0.059 Pr: 0.808 AIC: 15	d: 0.000 Pr: 0.868 AIC: -1381	d: 0.019 Pr: 0.982 AIC: 90
Decapod*area:volume [3]	d: 0.028 **Pr: <0.001** AIC: -485	d: 0.066 Pr: 0.829 AIC: 38	d: 0.000 Pr: 0.878 AIC: -1381	d: 0.02 Pr: 0.981 AIC: 90
Decapod*duration [3]	d: 0.03 **Pr: <0.001** AIC: -485	d: 0.065 Pr: 0.825 AIC: 34	d: 0.000 Pr: 0.875 AIC: -1381	d: 0.019 Pr: 0.981 AIC: 90
Decapod*trophic elements[3]	d: 0.022 **Pr: <0.001** AIC: -479	d: 0.065 Pr: 0.865 AIC: 58	d: 0.000 Pr: 0.904 AIC: -1381	d: 0.022 Pr: 0.978 AIC: 90

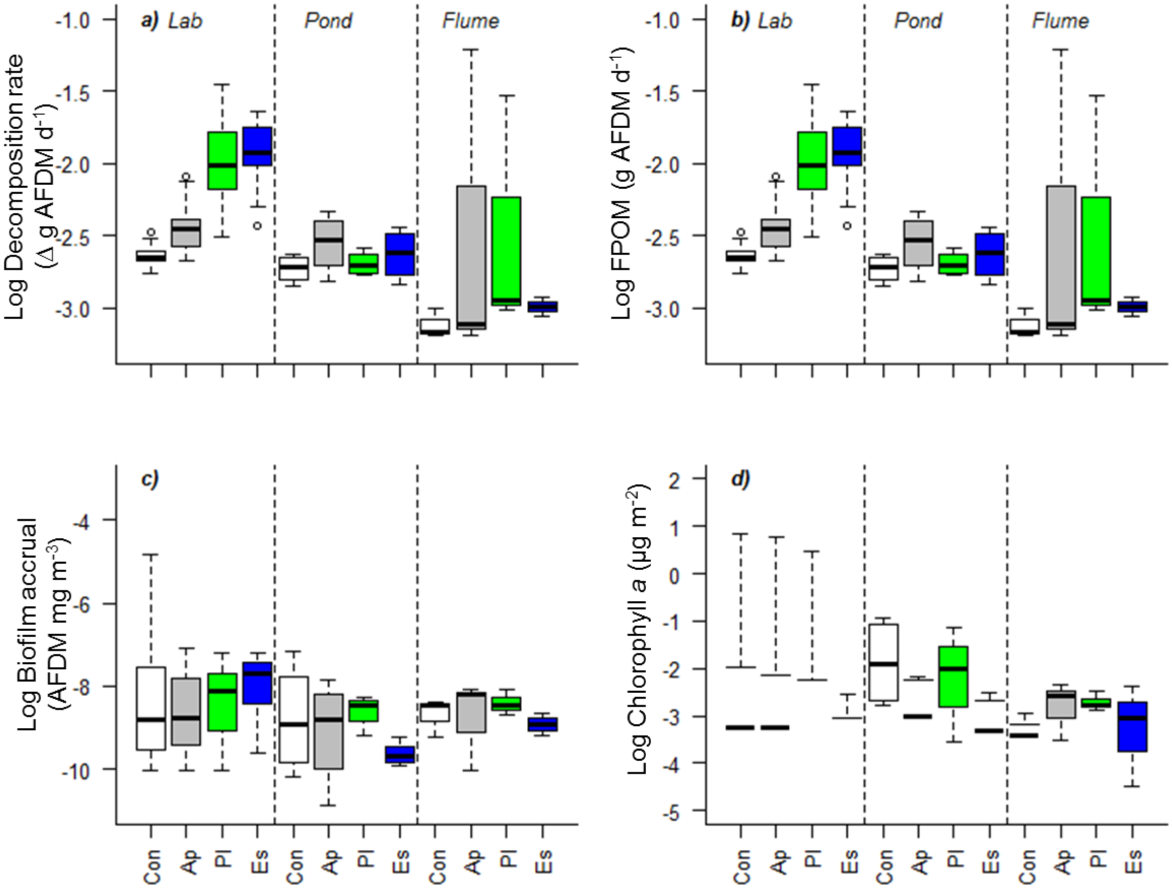

Figure 6.1. Ecosystem functioning variables across experimental scales (laboratory, pond mesocosm, flume mesocosm): leaf litter decomposition rate (Δ g AFDM d^{-1}) (a); production of fine particulate organic matter (FPOM g AFDM d^{-1}) (b); biomass accrual of biofilms (mg AFDM m^{-3}) (c); primary productivity of periphyton (chlorophyll *a* µg m^{-2}) (d). Variables are log transformed for graphical clarity. Abbreviation on x-axis: Con – Control; Ap – *Austropotamobius pallipes*; Pl – *Pacifastacus leniusculus* ; Es – *Eriocheir sinensis*.

Table 6.3. Generalized linear models assessing interactions between factorial decapod treatments and experimental venue for various water quality variables

Response variable	**Ammonia (mg L^{-1})**	**Nitrate (mg L^{-1})**	**Phosphate (mg L^{-1})**	**pH**
Factor [df] ↓				
Decapod species [3]	d: 0.012 Pr: 0.312 AIC: -341	d: 14.974 Pr: 0.841 AIC: 715	d: 0.069 Pr: 0.749 AIC: 1	d: 1.596 Pr: 0.755 AIC: 322
Experimental venue type [1]	d: 0.000 Pr: 0.958 AIC: -341	d: 898.98 **Pr: <0.001** AIC: 646	d: 5.254 **Pr: <0.001** AIC: -180	d: 81.814 **Pr: <0.001** AIC: 221
Venue area [1]	d: 0.000 Pr: 0.848 AIC: -341	d: 1738.600 **Pr: <0.001** AIC: 511	d: 4.329 **Pr: <0.001** AIC: -124	d:38.142 **Pr: <0.001** AIC: 284
Venue volume [1]	d: 0.000 Pr: 0.972 AIC: -341	d: 964.72 **Pr: <0.001** AIC: 639	d: 5.256 **Pr: <0.001** AIC: -180	d: 79.19 **Pr: <0.001** AIC: 226
Area:volume [1]	d: 0.000 Pr: 0.903 AIC: -341	d: 645.790 **Pr: <0.001** AIC: 668	d: 5.138 **Pr: <0.001** AIC: -171	d: 90.67 **Pr: <0.001** AIC: 201
Duration of experiment [1]	d: 0.000 Pr: 0.917 AIC: -341	d: 710.910 **Pr: <0.001** AIC: 663	d: 5.185 **Pr: <0.001** AIC: -175	d: 88.594 **Pr: <0.001** AIC: 206
No. trophic elements [1]	d: 0.000 Pr: 0.816 AIC: -342	d: 285.960 **Pr: <0.001** AIC: 695	d: 4.585 **Pr: <0.001** AIC: -137	d:98.745 **Pr: <0.001** AIC: 179
Decapod*venue type [3]	d: 0.012 Pr: 0.333 AIC: -337	d: 657.800 Pr: 0.978 AIC: 657	d: 0.028 Pr: 0.709 AIC: -119	d: 0.701 Pr: 0.716 AIC: 229
Decapod*venue area [3]	d: 0.006 Pr: 0.619 AIC: -335	d: 3.690 Pr: 0.797 AIC: 521	d: 0.028 Pr: 0.709 AIC: -119	d: 0.233 Pr: 0.972 AIC: 296
Decapod*venue volume [3]	d: 0.012 Pr: 0.355 AIC: -337	d: 1.820 Pr: 0.981 AIC: 651	d: 0.049 Pr: 0.257 AIC: -185	d: 0.676 Pr: 0.744 AIC: 235
Decapod*area:volume [3]	d: 0.014 Pr: 0.253 AIC: -337	d: 4.040 Pr: 0.958 AIC: 680	d: 0.048 Pr: 0.303 AIC: -174	d: 0.726 Pr: 0.630 AIC: 208
Decapod*duration [3]	d: 0.014 Pr: 0.273 AIC: -337	d: 3.440 Pr: 0.964 AIC: 674	d: 0.048 Pr: 0.281 AIC: -179	d: 0.732 Pr: 0.647 AIC: 213
Decapod*trophic elements [3]	d: 0.018 Pr: 0.156 AIC: -339	d: 9.205 Pr: 0.903 AIC: 706	d: 0.039 Pr: 0.5441 AIC: -135	d: 0.531 Pr: 0.663 AIC: 185

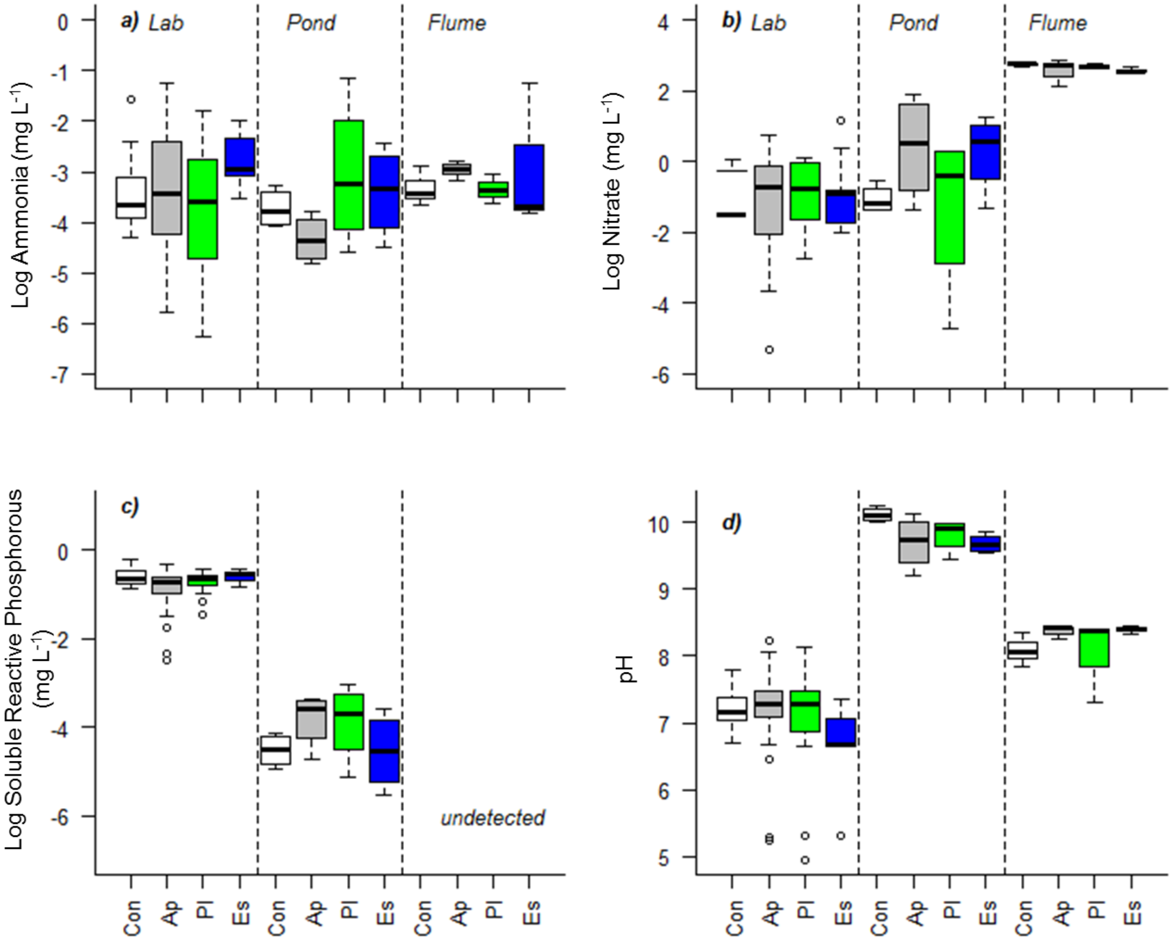

Figure 6.2. Water quality variables across experimental scales (laboratory, pond mesocosm, flume mesocosm): (a) ammonia (NH_4-N); (b) nitrate (NO_3-N) (c) soluble reactive phosphorous (PO_4-P); and (d) pH. Variables are log transformed for graphical clarity. X axis abbreviations are as for Fig 6.1.

6.3 Consequences of replacement of Austropotamobius pallipes *by invasive alien decapods*

Following the review of experimental scale and context and their importance when inferring the consequences of invasion by alien decapod species, the ecological consequences of the replacement of *A. pallipes* by invasive alien decapods are evaluated. The overall ecological impacts of *A. pallipes* compared to *P. leniusculus* and *E. sinensis* are summarised in Table 6.5. There was no consensus on each ecosystem attribute measured in these experiments, though non-redundancy was evident throughout the experimental venues even if this was not consistent.

Table 6.3. Summary of impacts of replacement of *Austropotamobius pallipes* by invasive alien decapods across multiple experimental venues.

Invasive alien species	**Ecosystem variable measured**	**Research venue**	**Outcome of invasion (- = negative impact; O = neutral; + = positive impact)**
Pacifastacus leniusculus	Leaf litter decomposition	Laboratory	+
		Mesocosm (pond)	O
		Mesocosm (flume)	O
	Biofilm (primary productivity)	Laboratory	O
		Mesocosm (pond)	+
		Mesocosm (flume)	O
	Sediment respiration	Mesocosm (pond)	O
		Mesocosm (flume)	+
	Chironomid larvae	Laboratory[1]	-
		Mesocosm (pond)	O
		Mesocosm (flume)	+
	Gastropod density	Laboratory[1]	-
		Mesocosm (pond)	-
		Mesocosm (flume)	O
	Shredder density	Laboratory[1]	-
		Mesocosm (pond)	O
		Mesocosm (flume)	O
Eriocheir sinensis	Leaf litter decomposition	Laboratory	+
		Mesocosm (pond)	O
		Mesocosm (flume)	O
	Biofilm (primary productivity)	Laboratory	O
		Mesocosm (pond)	O
		Mesocosm (flume)	O
	Sediment respiration	Mesocosm (pond)	O
		Mesocosm (flume)	O
	Chironomid density	Laboratory[1]	-
		Mesocosm (pond)	O
		Mesocosm (flume)	+
	Gastropod density	Laboratory[1]	-
		Mesocosm (pond)	-
		Mesocosm (flume)	O
	Shredder density	Laboratory[1]	-
		Mesocosm (pond)	O
		Mesocosm (flume)	O

[1] Laboratory results are from Haddaway et al. (2012) and Rosewarne et al. (2016)

From this study and that of others, it can be inferred that the invasion of *P. leniusculus* and *E. sinensis*, concurrently with the extirpation of *A. pallipes*, has the potential to alter the biodiversity and ecosystem functioning of river systems (see Fig 6.3), but that the response can be context-dependent. Both invasive alien decapods inflict a stronger top-down predation of snails than does the native species, which, at least in lentic water bodies, increases benthic algal productivity (Chapter 4). Bioturbation caused by burrowing, foraging and moving over the sediment was not clearly demonstrated, though *E. sinensis* induced heightened turbidity in lentic mesocosms, which potentially fed-back to reduce benthic primary productivity despite increased snail predation. However, bioturbation was not observed to be different between species in flume mesocosms, though the occurrence of existing refugia could have mediated this (Chapter 5). Sediment respiration was however greater in lotic mesocosms holding *P. leniusculus* than for *A. pallipes*, suggesting carbon cycling in rivers might be affected by this changeover of crayfish species. The processes responsible for this biogeochemical alteration require further study. This finding also assumes equal, moderate densities that are unrealistically low compared with some field observations (Clark et al., 1998, Nyström, 2002, Rudnick et al., 2003). Further experiments with greater densities of decapods could reveal even more significant effects on river carbon cycling processes.

Contrary to observations in published laboratory studies, chironomid larvae were either unaffected or increased in the presence of invasive alien decapod species (Chapter 4 & 5). This was partly explained by increased suspension of fine particulate organic matter in the water column (Chapter 2,3,5), which would provide a food source for these animals (Rosi-Marshall, 2004). The potential for nutrient recycling was found to be different between species, with *P. leniusculus* excreting less nitrogen than *A. pallipes* or *E. sinensis* (Chapter 2). This impact on dissolved nutrients was not observed in the laboratory, perhaps due to the presence of leaf-surface fungal and bacterial communities that would utilise such nutrients (Mehring et al., 2015). Similar patterns were observed in the lentic mesocosms, with the exception of lower nitrates for *P. leniusculus* (Chapter 4). While biofilms were productive in the presence of decapod predation on snails, this nutrient recycling did not feedback to productivity of biofilms in the laboratory. Further elemental analysis of biofilm C:N content (*sensu* Evans-White & Lamberti, 2005) could be investigated further for these species to assess whether biofilms are released from nutrient limitation.

The lower nitrogen excretion rate by *P. leniusculus* observed in Chapter 2 suggests this species could be a nutrient sink in aquatic ecosystems (*sensu* Vanni et al., 2013). These findings need further contextualisation, given that only sub-adult crayfish were measured (though these are usually the most dominant), and excretion can be dependent on the nutrient content of consumed resources (Vanni, 2002). Certain invasive alien species are hypothesised to be nutrient sinks, though these consist of species that are typically not consumed by native predators, such as armoured catfish (*Pterygoplichthys* sp.) or poisonous Cane Toads (*Rhinella marinus*) (Greenlees et al., 2006, Capps and Flecker, 2013). However, *P. leniusculus* and other invasive alien crayfish are known to be consumed by native European predators, including birds of prey, herons and otter (Wood et al., 2016, Mortimer et al., 2012, Tablado et al., 2010) which may redistribute nutrients outside of aquatic systems. Predation rates upon *P. leniusculus* by other species in the ecosystem such as fish, birds and mammals will need to be considered to understand the mass-balance of nutrients and biomass within an invaded ecosystem. This could be uncertain in the context of fish predators, as crayfish injure fish (Zhang et al., 2004) as well as predate their eggs and fry (Edmonds et al., 2011), and headwater streams can undergo dramatic declines in recruitment of fish following the replacement of *A. pallipes* by more dense populations of *P. leniusculus*. This could be verified through monitoring populations of crayfish and their potential predators in progressively invaded river and lakes, or in large mesocosms. Management interventions could also take place where potential predators are monitored before, during and after invasive alien decapod control.

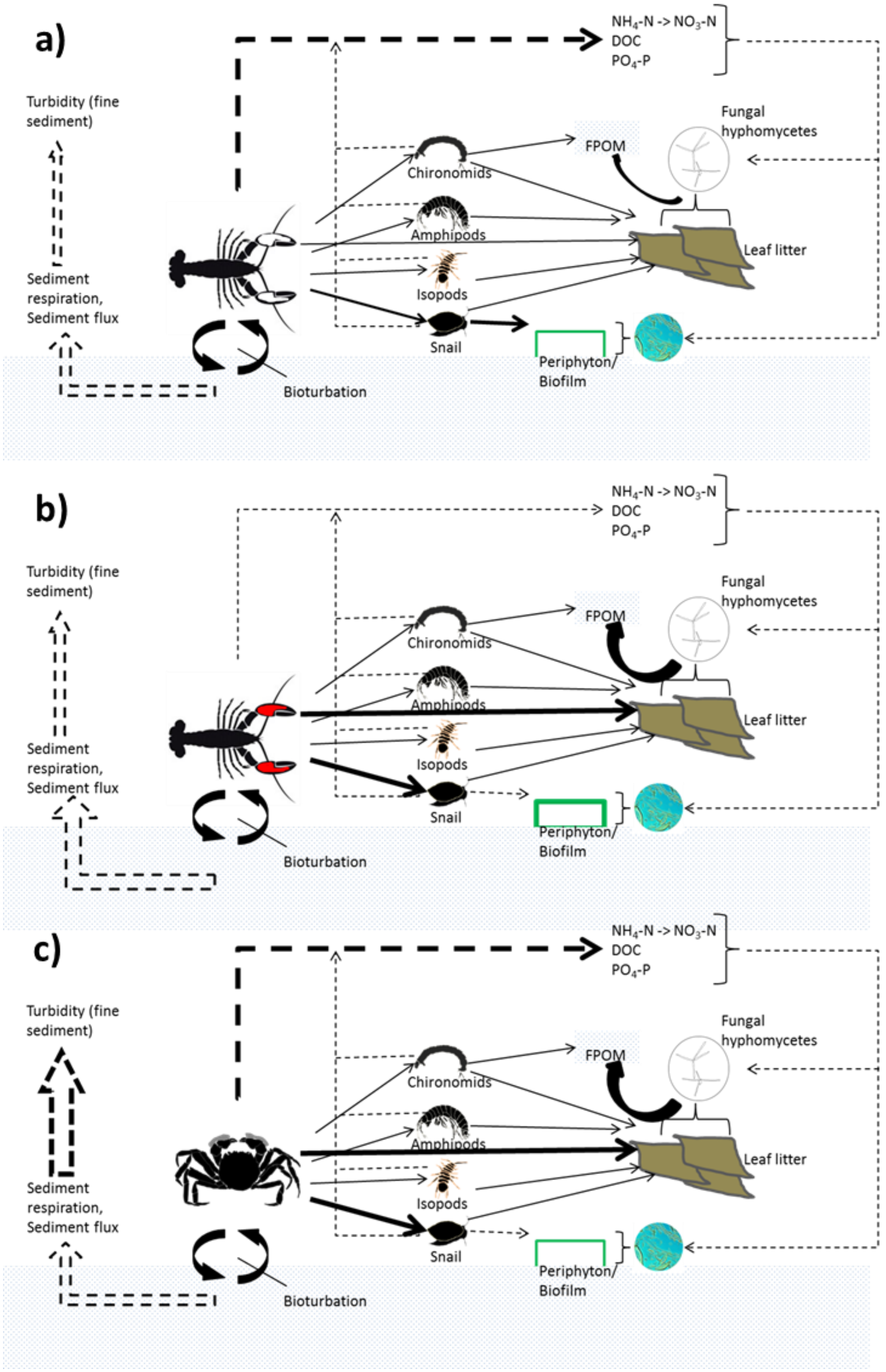

Figure 6.3. A basic functioning ecosystem summary of updated working hypotheses on the ecological impacts of a) *Austropotamobius pallipes*, b) *Pacifastacus leniusculus* and c) *Eriocheir sinensis* on freshwater ecosystems, based on the results of this thesis and other studies cited therein. Relative size of the arrow indicates differences in impact between species.

It is currently assumed that *A. pallipes* will be replaced by *E. sinensis* when the two species interact, but this is currently uncertain. *E. sinensis* was predicted to negatively impact *A. pallipes* populations by Clark et al. (1998). *E. sinensis* has colonised rivers formerly inhabited by the native crayfish, though only after the native had been replaced by other crayfish species (Almeida et al., 2014). Interactions between *E. sinensis* and *A. pallipes* however have not been researched, but *E. sinensis* is known to asymptomatically carry Crayfish Plague, and could cause declines of *A. pallipes* (Schrimpf et al., 2014), though the prevalence of this pathogen in *E. sinensis* populations has not been surveyed. In the absence of invasive alien crayfish in Ireland, *E. sinensis* is likely to interact with *A. pallipes* in isolation, and could provide an opportunity to understand the outcomes for this otherwise uncertain interspecific interaction. This opportunity would preferably be avoided, with preventative measures currently being encouraged (Kelly and Maguire, 2009). Experiments investigating the interactions between these two species would thus be more appropriate, notably shelter use, and antagonistic interactions, similar to those of Bubb et al. (2006) and Gilbey et al. (2008).

While *P. leniusculus* is the prominent invasive alien decapod impacting *A. pallipes* in north-western Europe, in the long term, without successful interventions, other decapod species could be more important for the regulation novel freshwater ecosystems. The Virile Crayfish (*Oronectes virilus*) from North America has recently been found to outcompete *P. leniusculus* in laboratory arena interactions, and to replace it entirely in rivers where is was previously dominant (James et al., 2016). *O. virilus* has already been found to have a greater, negative impact on grazing invertebrates, decomposition rates, and cascading increase of periphyton productivity than *P. leniusculus* (Jackson et al., 2014). This highlights the dynamic and unpredictable nature of biological invasions in the uncertainty they bring, but also how results from in-depth mesocosm experiments, such as those employed in this thesis, can help resolve this uncertainty.

The biodiversity and ecosystem functioning of rivers, streams, lakes, ponds and canals invaded by both *P. leniusculus* and *E. sinensis* are predicted to be altered when they replace *A. pallipes* (Fig 6.3, Table 6.3). Further work to replicate these results should consider repeating the mesocosm experiments studies using higher densities of decapods, including representative demographics (such as deployed by Nyström et al., 2001). *In situ* studies of ecological impacts could utilise natural experiments of whole ecosystems that are either uninvaded, progressively invaded and established invasion, which provides the advantage of long term data in real world scenarios (Strayer et al., 2006, Strayer, 2012).

6.4 Implications for management of invasive decapod invasions

A significant management implication of this research is that certain ecosystem responses to biological invasions might not scale from the laboratory to the field. Managers of biological invasions therefore need to be careful in interpreting ecological experiments that might be limited in relevance to real-world scenarios. Some results from the mesocosms do have implications for management of river ecosystems though, where habitats of native crayfish invaded by alien decapods can undergo changes to biodiversity, ecosystem properties and functioning. The variation of responses in the experimental venues indicate there could be a similar variation in effect depending on the ecosystem type/context (e.g. river vs lake, location in river network, history of disturbance). This has implications for interpretations of impacts that would motivate interventions such as investment in prevention, control and attempted eradication. The more realistic mesocosm experiments suggested that while fundamental changes to ecosystems can occur due to the novel functional traits of the invasive alien species, many properties and processes (such as biodiversity, detrital processes, gross primary productivity) appeared to be resilient to the invasion. This would be contingent on densities of the invading decapods being equivalent to the native analogue they replace. As these functional differences could be amplified by differences in densities to impact ecosystems, the results of the experiments described in this thesis suggest that if populations of the invaders could at least be regulated, effects on ecosystem properties (including biodiversity) and processes could be minimised.

The regulation of invasive alien decapod populations is not well studied, and control efforts of established populations are still in the experimental phases. These include promotion of natural crayfish predators, use of biocides, trapping and removal of crayfish and sterilization of males to disrupt breeding recruitment (Stebbing et al., 2003, Peay et al., 2006, Aquiloni et al., 2010). Trap and removal of invasive alien crayfish (including *P. leniusculus*) has so far found positive effects for invertebrate diversity (Hansen et al., 2013a, Moorhouse et al., 2014) and to reduce the body condition of invasive alien crayfish (demonstrating selection pressure on the population) (Moorhouse and Macdonald, 2011). As trapping crayfish and crabs to control numbers is labour intensive, current techniques do not remove representative proportions of juveniles and would require substantial, sustained investment, protecting native ecosystems via this approach is uncertain and therefore likely to be unfeasible at present. So far, only collection of *E. sinensis* for human consumption has been proposed for its control (Clark, 2011), with prevention through the treatment of shipping ballast water advocated (Hulme, 2015). Mitigating against the decline of *A. pallipes* has so far been restricted to captive breeding, creating barriers to invasive crayfish colonization and translocation to crayfish-free habitats that are difficult to access naturally by other crayfish (Kozák et al., 2011). However, these translocation have the potential to negatively impact biodiversity and ecosystem processes through *A. pallipes* predating invertebrates, fish eggs, amphibians and altering sediments (Gherardi et al., 2001, Edmonds et al., 2011, Rosewarne et al., 2013, James et al., 2015, Chapter 4 & 5).

While control of established invasive alien decapods is still uncertain, a manager of an invaded aquatic habitat that might previously have supported *A. pallipes* would possibly be faced with an altered ecosystem, and thus mandated to mitigate the undesirable changes. Should direct management of decapod populations not be feasible, other interventions such as encouraging populations of decapod predators might help regulate invasive decapod populations (Whitehouse et al., 2009). However, given *A. pallipes* has persisted with these same native predators since the Pleistocene, it is unlikely invasive alien decapods would be extirpated by natural enemies. If not managing populations of invasive alien decapods, their effects on ecosystem processes could be mitigated. For example, refugia such as woody debris and boulders could be strategically placed around river basins to reduce sediment disturbance, and to capture transported sediments. Structures to enable refuge to vulnerable species, such as snails, to persist without complete extirpation by decapods could also be created, though the challenge of this would be to prevent access by all age-classes of crayfish or crab. Prevention of other disturbances, such as chemical pollution could maximise the resilience of freshwater ecosystems to invasions through compensatory effects by species not affected by the invasion. Invasive alien decapods could bring potential positive impacts, including the regulation of the impacts of other harmful invasive alien species, such as reducing the accumulation of otherwise unpalatable leaf litter (Chapter 3). While management interventions for invasive alien species measure their effectiveness through either outcomes of invasion success or density of target species (Simberloff et al., 2012a), few consider impacts on ecosystem properties and processes (Strayer, 2012), as measured in this study. Thus, it is proposed that in addition to monitoring occurrences and densities of invasive alien species in freshwaters, variables such as invertebrate diversity and density, gross primary productivity, suspended organic matter, periphyton productivity, leaf litter processing rates, nutrient levels and general water quality also be measured to assess impacts and any response to management interventions.

6.5 Concluding remarks

This thesis has demonstrated that previously published laboratory measurements of predatory functional responses that show non-redundancy of invasive alien decapods can predict impacts on other resources, but not consistently across ecosystem attributes or contexts. The impact of ecosystem complexity on the impact of invasive alien species and resilience of ecosystems to invasions has been indicated from this work. Key findings from this thesis include:

- The invasive alien decapods *P. leniusculus* and *E. sinensis* breakdown leaf litter at a greater rate than *A. pallipes* in the absence of other invertebrate prey, including the transformation into other materials that other species might consume (FPOM) (Chapter 2). This included leaf litter that is not readily processed by native detritivore communities (Chapter 3).
- Invasive alien decapods can have differential nutrient excretion to native species, which has the potential to impact nutrient cycling, though the potential to be a nutrient sink is unknown with regard to predation by other, larger species (Chapter 2 & 6).
- Invasive alien decapods had a greater impact on snails than native crayfish in pond experiments, causing a trophic cascade that increased periphyton productivity (Chapter

4). This effect on periphyton was however dampened by presumed bioturbatory effects of decapods reducing light penetration, thus photosynthesis in freshwater ecosystems.

- Invasive alien decapods had different, positive impacts on invertebrate taxonomic diversity compared to *A. pallipes*, explained by the facilitation of species that would consume particulate organic matter (Chapter 4 & 5).
- Impacts on sediment topology and transport by all decapod species were not observed in the presence of refuges. In scenarios where river and lake beds are homogenized, it might be predicted that decapods could impact sediments following removal of natural refugia (Chapter 5).
- Effects of invasive alien decapods were not consistent when appraised at different scales and contexts (Chapter 6).
- The overall impacts of replacement of *A. pallipes* by *P. leniusculus* and *E. sinensis* are predicted to be non-redundant and context dependent. Furthermore, the effects demonstrated in the thesis are all per capita. In field situations, likely changes to certain ecosystem services will be amplified by the greater size and density differences.

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Supplementary material

Supplementary Table 2.1. Tukey test showing pair-wise comparisons of treatments that showed significant relationships based on a Generalized Linear Model

Response variable	**Treatment**	**Tukey test p-value**			
		Control	Ap-p	Ap	Pl
Decomposition rate	Control	-			
	Ap-p	0.687	-		
	Ap	0.121	0.978	-	
	Pl	**<0.001**	**<0.001**	**<0.001**	-
	Es	**<0.001**	**<0.001**	**<0.001**	0.794
Detritivory performance	Control	-			
	Ap-p	-	-		
	Ap	-	0.861	-	
	Pl	-	0.541	0.899	-
	Es	-	**0.021**	**0.048**	0.186
CPOM (10-1 mm) Production	Control	-			
	Ap-p	0.999	-		
	Ap	0.936	0.936	-	
	Pl	**<0.001**	**<0.001**	**<0.001**	-
	Es	**<0.001**	**<0.001**	**<0.001**	0.818
FPOM (1mm-0.7µm) Production	Control	-			
	Ap-p	0.999	-		
	Ap	0.784	0.967	-	
	Pl	**<0.001**	**<0.001**	**<0.001**	-
	Es	**<0.001**	**<0.001**	**<0.001**	0.937
FPOM production by decapod mass	Control	-			
	Ap-p	-	-		
	Ap	-	0.911	-	
	Pl	-	**<0.001**	**<0.001**	-

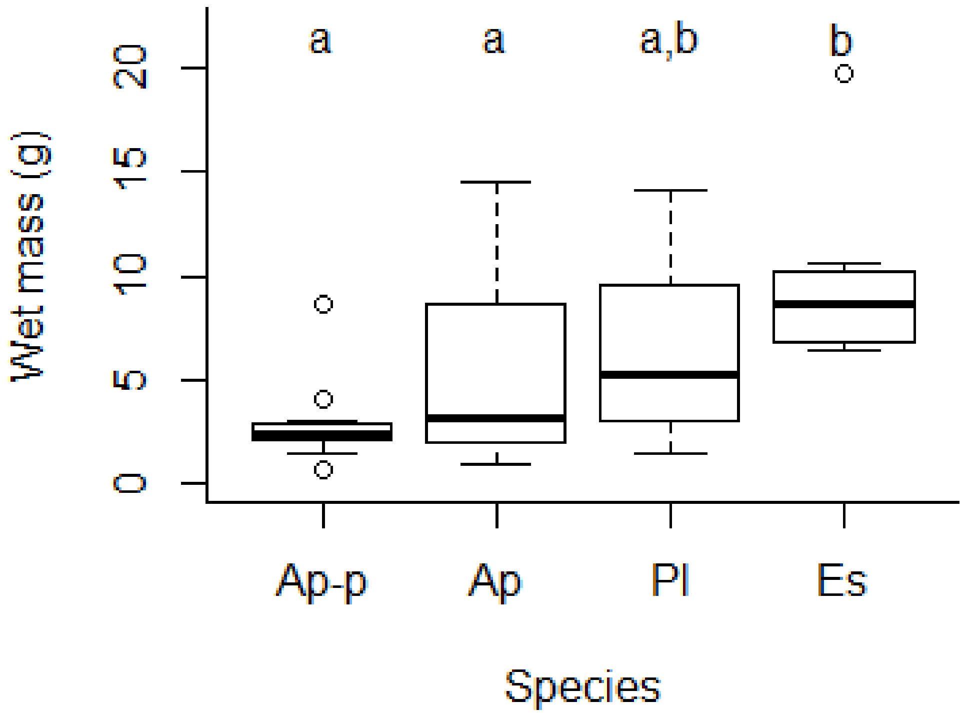
	Es	-	0.100	0.178	0.083
DOC Production	Control	-			
	Ap-p	0.997	-		
	Ap	0.999	1.000	-	
	Pl	0.167	0.604	0.278	-
	Es	0.060	0.319	0.106	0.947
DOC production efficiency	Control	-			
	Ap-p	-	-		
	Ap	-	0.976	-	
	Pl	-	0.233	0.251	-
	Es	-	**0.012**	**0.012**	0.27
Soluble Reactive Phosphorous	Control	-			
	Ap-p	**<0.001**	-		
	Ap	0.313	**0.035**	-	
	Pl	0.473	**0.018**	0.998	-
	Es	1.000	**<0.001**	0.431	0.577

Supplemental Table 2.2. *Post-hoc* Tukey tests for generalized linear models for nutrient excretion between controls and decapod species

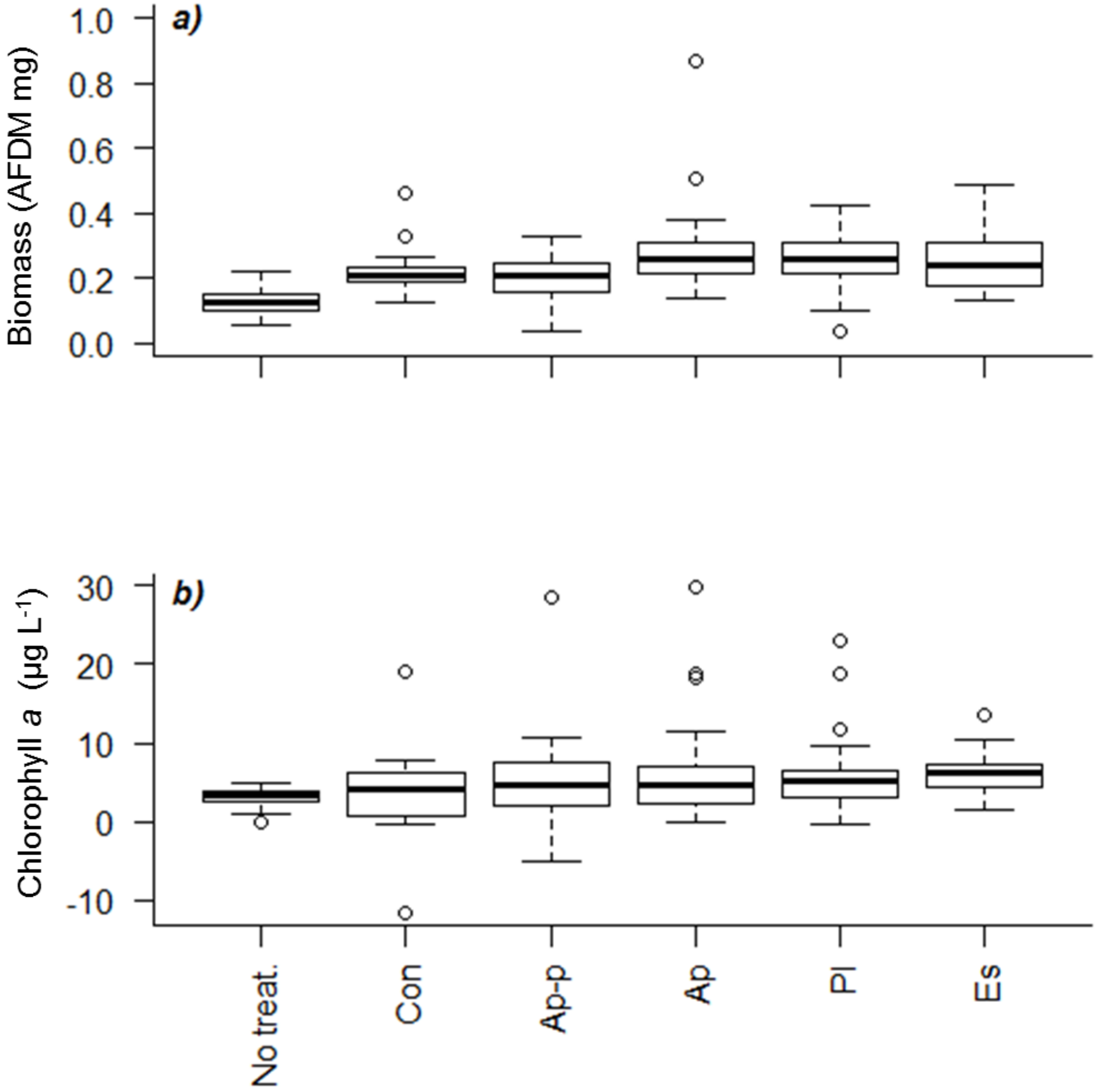
Nutrient	Treatment	**Tukey test p-value**		
Ammonia (NH_3-N)		Con	Ap	Pl
	Con	-		
	Ap	**0.001**	-	
	Pl	0.999	**0.004**	-
	Es	**<0.001**	0.818	**<0.001**
Nitrate (NO_3-N)	Con	-		
	Ap	**<0.001**	-	
	Pl	**<0.001**	0.988	-
	Es	**<0.001**	0.940	0.819
Soluble reactive phosphorous (PO_4-P)	Con	-		
	Ap	0.321	-	
	Pl	0.844	0.839	-
	Es	0.007	0.392	0.089

Supplemental Table 2.3. Correlates of biofilms and detrital processing

Type of biofilm	Response variable	Explanatory factor	d.f.	Deviance residuals	Pr(>Chi)	R^2
Colonised tiles	Total biomass	Decomposition rate	1	0.0002	**0.04**	0.03
		FPOM	1	0.0001	0.08	n/a
		DOC	1	0.0001	0.07	n/a
		NH_4-N	1	0.00005	0.31	n/a
		NO_3-N	1	0.00004	0.34	n/a
		PO_4-P	1	0.00009	0.15	n/a
New biofilm accrual (blank tiles)	Total biomass	Decomposition rate	1	0.00009	0.85	n/a
		FPOM	1	0.00001	0.93	n/a
		DOC	1	0.00001	0.93	n/a
		NH_4-N	1	0.00002	0.90	n/a
		NO_3-N	1	0.01	**0.002**	0.04
		PO_4-P	1	0.003	0.18	n/a
Colonised tiles	Primary productivity	Decomposition rate	1	0.09	0.68	n/a
		FPOM	1	0.16	0.59	n/a
		DOC	1	0.01	0.89	n/a
		NH_4-N	1	0.09	0.68	n/a
		NO_3-N	1	0.11	0.66	n/a
		PO_4-P	1	2.07	0.05	0.03
New biofilm accrual (blank tiles)	Primary productivity	Decomposition rate	1	0.10	0.40	n/a
		FPOM	1	0.009	0.81	n/a
		DOC	1	0.02	0.72	n/a
		NH_4-N	1	0.20	0.25	n/a
		NO_3-N	1	0.04	0.59	n/a
		PO_4-P	1	0.37	0.12	n/a



Supplementary Figure 2.1. Distribution of mass of the different decapod species used in the experiment.



Supplementary Figure 2.2. a) Biomass accrual (mg ash free dry mass) and b) primary productivity (chlorophyll *a*) of established biofilms. Abbreviations as with Fig. 1, with "no treat." Indicating tiles sampled before the experiment to highlight growth.

Supplementary Table 3.1. *Post hoc* Tukey test results for leaf litter decomposition rate

	Control	Ap	Pl
a) Alder			
Control	-		
Ap	0.349	-	
Pl	**<0.001**	**0.015**	-
Es	**<0.001**	**0.002**	0.924
b) Sycamore			
	Control	Ap	Pl
Control	-		
Ap	**0.007**	-	
Pl	**<0.001**	**<0.001**	-
Es	**<0.001**	**<0.001**	0.999
c) Rhododendron			
	Control	Ap	Pl
Control	-		
Ap	0.107	-	
Pl	**<0.001**	**0.004**	-
Es	**0.001**	0.382	0.247

Supplementary Table 3.2. *Post hoc* Tukey test results for production of CPOM

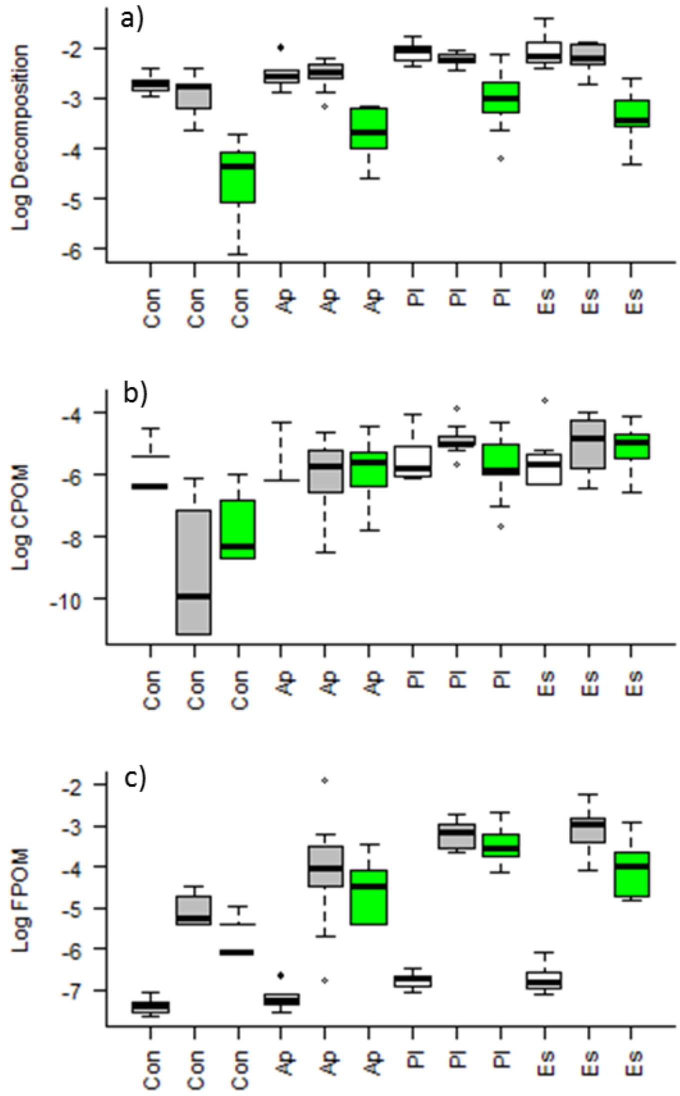
	Control	Ap	Pl
a) Alder			
Control	-		
Ap	0.964	-	
Pl	0.805	0.555	-
Es	0.730	0.480	0.999
b) Sycamore			
Control	-		
Ap	0.368	-	
Pl	**0.003**	0.076	-
Es	**<0.001**	**0.011**	0.854
c) Rhododendron			
Control	-		
Ap	**0.049**	-	
Pl	**0.014**	0.884	-
Es	**0.001**	0.197	0.568

Supplementary Table 3.3. *Post hoc* Tukey test results for production of FPOM

	Control	Ap	Pl
a) Alder			
Control	-		
Ap	0.349	-	
Pl	**< 0.001**	**0.015**	-
Es	**< 0.001**	**0.002**	0.924
b) Sycamore			
	Control	Ap	Pl
Control	-		
Ap	**0.006**	-	
Pl	**<0.001**	**0.010**	-
Es	**<0.001**	**0.014**	0.998
c) Rhododendron			
	Control	Ap	Pl
Control	-		
Ap	**0.025**	-	
Pl	**<0.001**	**0.002**	-
Es	**0.001**	0.369	0.121

Supplementary Table 3.4. Post hoc Tukey test for change in decapod mass across different leaf species

Decapod sp.		Alder	Sycamore
A. pallipes	Alder	-	
	Sycamore	0.185	-
	Rhododendron	0.705	0.745
P. leniusculus	Alder	-	
	Sycamore	**0.040**	-
	Rhododendron	**0.007**	0.609
E. sinensis	Alder	**-**	
	Sycamore	0.528	-
	Rhododendron	0.611	**0.040**



Supplemental Figure 3.1. Raw detrital processing variables not divided by decapod mass, showing values for decapod-free controls.

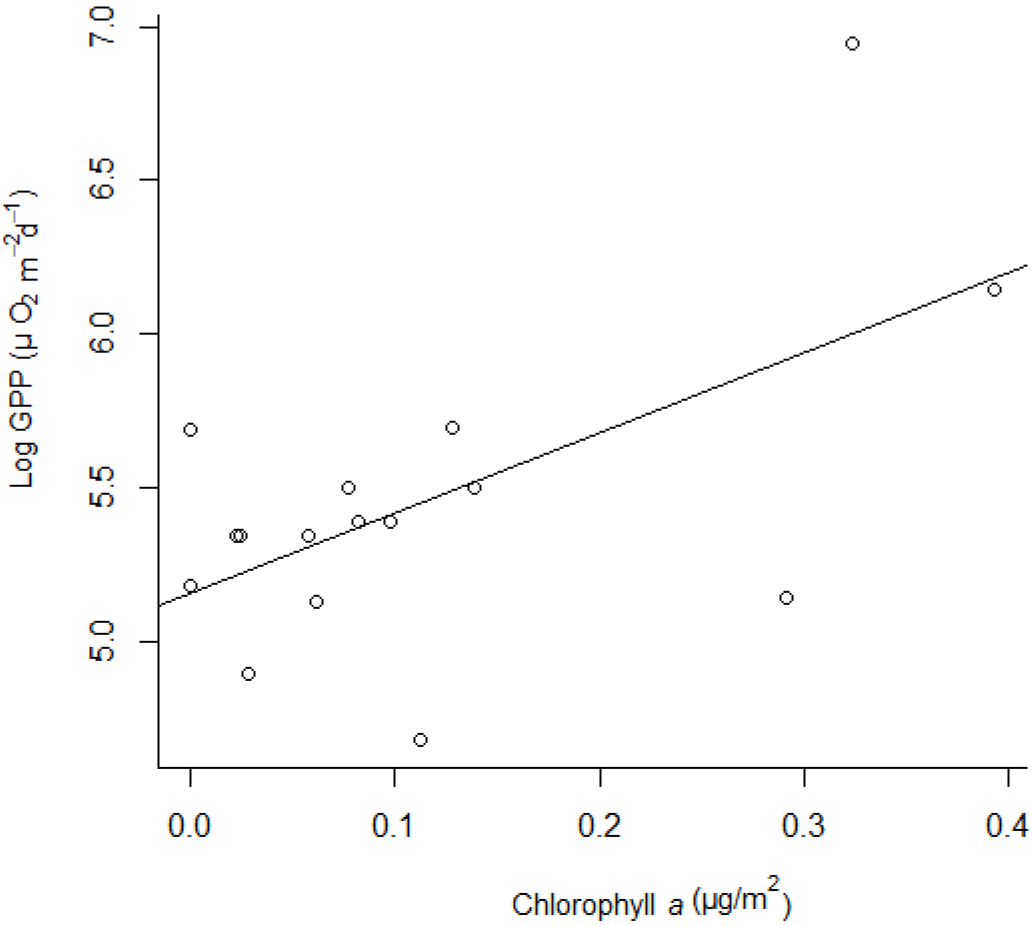
Supplementary Table 4.1. Macroinvertebrate inventory and origin of mesocosms

Taxon	**Origin**
Acari	Colonised
Agabus biguttatus	Colonised
Agabus nebulosi	Colonised
Agabus unguiculuois/ Ilybio sp.	Colonised
Apatania sp.	Colonised
Asellus aquaticus	Seeded
Baetis sp.	Seeded
Bithnyia tentaculata	Seeded
Ceratopogoninae	Colonised
Chaoborus sp.	Colonised
Chironomidae	Both
Coenagrionidae	Colonised
Culex sp.	Colonised
Dasyhelea	Colonised
Elmidae	Colonised
Elmis sp.	Colonised
Ephydridae	Colonised
Gammarus pulex	Seeded
Glossiphonia sp.	Colonised
Halipus linealatis	Colonised
Heloporous sp.	Colonised
Hydroporous sp.	Colonised
Hyphydrus aubei	Colonised
Hyrdrophilidae	Colonised
Libellulidae	Colonised
Limnebius sp.	Colonised
Limnephllidae	Colonised
Lymnaea palustris	Seeded
Lymnaea stagnalis	Seeded
Lymnius type Elmidae	Colonised
Micronecta sp.	Colonised
Nebrioporus depressus	Colonised
Nemertea	Colonised
Notonecta sp.	Colonised
Odontocercum albicone	Colonised
Oligochaeta	Colonised
Physa frontinalis	Seeded
Planarian	Colonised
Planorbis albus	Seeded
Planorbis corneus	Seeded
Planorbis vortex	Seeded

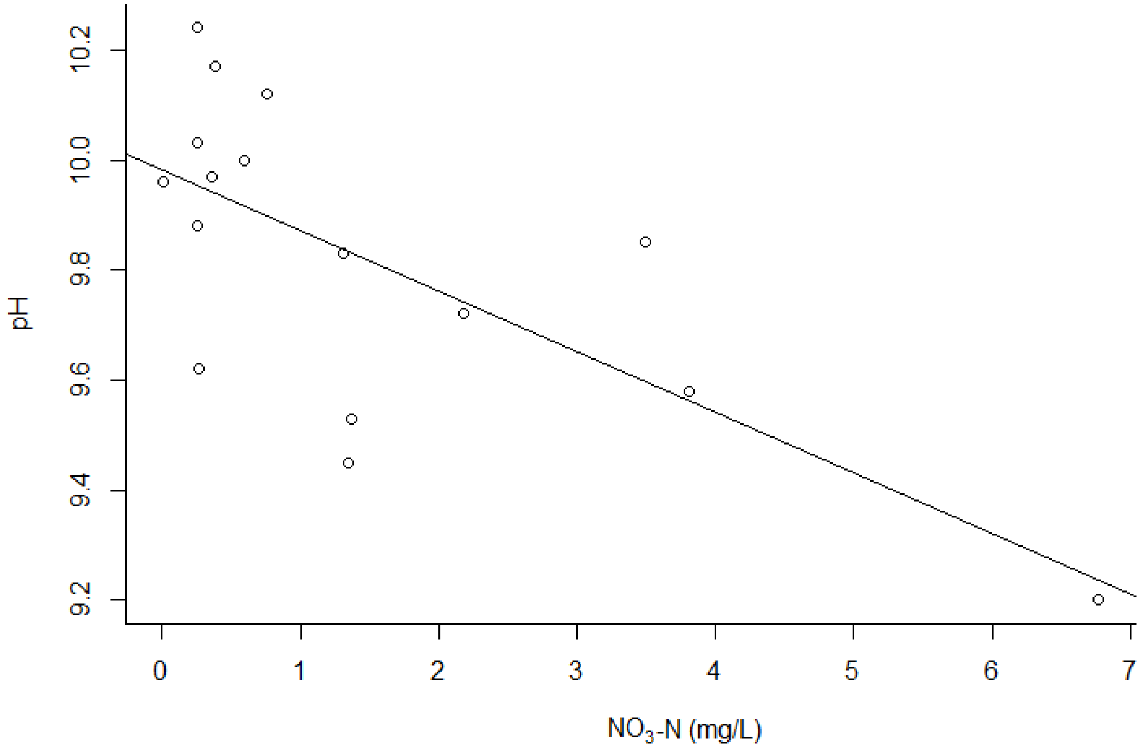
Platycemis sp.	Colonised
Radix peregra	Seeded
Rhantus grapii	Colonised
Sericostoma sp.	Seeded
Strictotarsus duodecimpast-ulatus	Colonised
Tipulidae	Colonised

Supplemental Table 4.2. Water physiochemical parameters

Parameter	Units	Mean	StDev
Dissolved oxygen	mg L^{-1}	12.27	1.82
Ammonia (NH_4-N)	mg L^{-1}	0.05	0.08
Nitrate (NO_3-N)	mg L^{-1}	1.46	1.82
pH	-	9.82	0.29
Soluble reactive phosphorous	mg L^{-1}	0.02	0.01
Water temperature	Celsius	23.1	0.8



Supplemental Figure 4.1. Correlation of gross primary productivity of mesocosms and primary productivity of biofilms (measured using chlorophyll *a*).



Supplemental Figure 4.2. Correlation of pH and nitrates in the mesocosms.

Supplemental Table 5.1. Results of SIMPER analysis

Taxon	**Control**		**A. pallipes**			
	Density (individuals/m^2)	**StDev**	**Density (individuals/m^2)**	**StDev**	**Contribution to dissimilarity**	**Cumulative contribution**
Chironomidae	224.81	81.29	169.63	29.45	0.14	0.54
Gammarus	75.00	28.33	54.26	24.43	0.05	0.75
Baetis	13.70	20.46	5.37	8.34	0.03	0.86
Dasyhelea	6.11	6.41	0.00	0.00	0.01	0.90
Potamopyrgus	3.52	2.31	2.78	3.47	0.00	0.92

	Control		***P. leniusculus***			
	Density (individuals/m^2)	**StDev**	**Density (individuals/m^2)**	**StDev**	**Contribution to dissimilarity**	**Cumulative contribution**
Chironomidae	224.81	81.29	275.00	210.43	0.19	0.60
Gammarus	75.00	28.33	62.78	37.17	0.05	0.75
Baetis	13.70	20.46	40.74	10.63	0.04	0.88
Dasyhelea	6.11	6.41	2.22	2.55	0.01	0.91
Culicoides	0.19	0.32	5.19	3.06	0.01	0.93

	Control		***E. sinensis***			
	Density (individuals/m^2)	**StDev**	**Density (individuals/m^2)**	**StDev**	**Contribution to dissimilarity**	**Cumulative contribution**
Chironomidae	224.81	81.29	362.22	230.03	0.17	0.52
Baetis	13.70	20.46	115.00	85.73	0.10	0.82
Gammarus	75.00	28.33	75.19	20.56	0.03	0.91
Dasyhelea	6.11	6.41	4.44	3.38	0.01	0.93
Culicoides	0.19	0.32	3.15	4.52	0.00	0.95

	A.pallipes		***P. leniusculus***			
	Density (individuals/m^2)	**StDev**	**Density (individuals/m^2)**	**StDev**	**Contribution to dissimilarity**	**Cumulative contribution**
Chironomidae	169.63	29.45	275.00	210.43	0.15	0.52
Baetis	54.26	24.43	40.74	10.63	0.06	0.71
Gammarus	5.37	8.34	62.78	37.17	0.05	0.88
Culicoides	0.37	0.64	5.19	3.06	0.01	0.91
Potamopyrgus	2.78	3.47	1.85	1.28	0.00	0.92

	A.pallipes		***E. sinensis***			
	Density (individuals/m^2)	**StDev**	**Density (individuals/m^2)**	**StDev**	**Contribution to dissimilarity**	**Cumulative contribution**
Chironomidae	169.63	29.45	362.22	230.03	0.20	0.51
Baetis	54.26	24.43	115.00	85.73	0.12	0.83
Gammarus	5.37	8.34	75.19	20.56	0.04	0.93
Dasyhelea	0.00	0.00	4.44	3.38	0.01	0.94
Potamopyrgus	2.78	3.47	1.85	1.70	0.00	0.95

	P. leniusculus		***E. sinensis***			
	Density (individuals/m^2)	**StDev**	**Density (individuals/m^2)**	**StDev**	**Contribution to dissimilarity**	**Cumulative contribution**
Chironomidae	275.00	210.43	362.22	230.03	0.21	0.61
Baetis	40.74	10.63	115.00	85.73	0.07	0.81
Gammarus	62.78	37.17	75.19	20.56	0.04	0.92
Culicoides	5.19	3.06	3.15	4.52	0.00	0.94
Dasyhelea	2.22	2.55	4.44	3.38	0.00	0.95