The effects of agricultural land use on the community structure and functioning of small freshwater habitats

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To my husband, Omar, for his endless support and great help through my PhD…To my lovely daughters, Jana & Mona, for their patience…To my parents who never stop supporting me.

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Abstract

Agriculture is important for maintaining human well-being, but intensive agricultural production can have adverse environmental impacts. Freshwater ecosystems are particularly vulnerable to chemical and physical stressors resulting from agricultural land use, but few studies have compared the effects of different types of agricultural practices on the ecology of small water bodies. In agricultural catchments, the two major land uses, arable and pastoral, differ in their management (e.g. ploughing, fertilizer and pesticide inputs) and therefore in their potential effect on freshwater communities. The aim of this study was to investigate the effects of two types of agricultural land use (arable and pastoral) on the community structure and functioning of ponds and streams. Twenty four sites (6 arable ponds, 6 arable streams, 6 pastoral ponds and 6 pastoral streams) were studied in Leicestershire, UK. Arable streams had lower macroinvertebrate abundance and taxonomic richness than pastoral streams and arable ponds had lower diatom species richness, diversity and evenness than pastoral ponds. Leaf litter decomposition was also higher in arable than pastoral ponds. Feeding by macroinvertebrate shredders (e.g. *Gammarus pulex*) was a significant contributor to leaf breakdown in streams but not in ponds. The feeding rates of *G. pulex* and *Asellus aquaticus* were significantly affected by temperature and intraspecific interactions (i.e. density). Increasing density resulted in greater per capita leaf mass loss of *A. aquaticus* and lower survival rate of *G. pulex*. At higher temperature, the per capita leaf mass loss and feeding rates for both species were greater whereas the survival rates were lower. Agricultural land use can adversely affect the structure and functioning of aquatic communities. Consequently, it may have a considerable potential impact on ecosystem services provided by freshwater habitats. Understanding the possible effects of agricultural land use on the structure and functioning of freshwater ecosystems is extremely important and should help in identifying the best land use management to maintain sustainable agricultural production and protect freshwater habitats.

Table of Contents

Acknowledgements iii
Abstract iv

Chapter 1: General introduction 1
- 1.1. Introduction 1
- 1.2. Intensification of agriculture 3
- 1.3. Ecosystem services provided by agricultural habitats 6
  - 1.3.1. The role of biodiversity in delivering ecosystem services 8
- 1.4. Agricultural landscapes in the UK 10
- 1.5. Aim and objectives 13

Chapter 2: Study site descriptions 16
- 2.1. Site selection 18
- 2.2. Site-specific land use, elevation and flow patterns 25
- 2.3. Physicochemical characteristics of water 33
- 2.4. Conclusion 36

Chapter 3: The effects of different types of agricultural land use on freshwater macroinvertebrate communities 37
- 3.1. Introduction 37
- 3.2. Methods 40
  - 3.2.1. Study sites 40
  - 3.2.2. Macroinvertebrate sorting and identification 40
  - 3.2.3. Data analysis 41
- 3.3. Results 42
  - 3.3.1. Physicochemical characteristics of freshwater habitats 42
  - 3.3.2. Total abundance, taxonomic richness and diversity of macroinvertebrates 44
  - 3.3.3. Relative abundance of major macroinvertebrate groups 47
  - 3.3.4. Macroinvertebrate community composition 52
- 3.4. Discussion 55
- 3.5. Conclusion 59

Chapter 4: Leaf litter decomposition in agricultural ponds and streams located in different types of land use 60
4.1. Introduction 60
4.2. Methods 64
  4.2.1. Study sites 64
  4.2.2. Leaf decomposition 65
  4.2.3. In situ feeding rate 66
    4.2.3.1. Fungus culture preparation 66
    4.2.3.2. Preparing leaf discs 66
    4.2.3.3. Animal collection and maintenance 67
    4.2.3.4. Field deployment 67
    4.2.3.5. Water chemistry 68
  4.2.4. Data analysis 68
4.3. Results 70
  4.3.1. Leaf decomposition 70
  4.3.2. Macroinvertebrates in leaf bags 74
  4.3.3. Water chemistry 83
  4.3.4. In situ feeding rate 85
4.4. Discussion 87
4.5. Conclusion 90

Chapter 5: The impacts of agricultural land use on algal communities 92
5.1. Introduction 92
5.2. Methods 97
  5.2.1. Study sites 97
  5.2.2. Sampling algal communities 97
  5.2.3. Chlorophyll *a* analysis 97
  5.2.4. Diatom preparation and identification 98
  5.2.5. Data analysis 99
5.3. Results 101
  5.3.1. Chlorophyll *a* concentration 101
  5.3.2. Diatom species richness, diversity, evenness and TDI 102
  5.3.3. The relationship between nutrients and chlorophyll *a* and TDI 107
  5.3.4. Diatom community composition 110
5.4. Discussion 114
5.5. Conclusion 117

Chapter 6: The effect of intraspecific interactions on the feeding rate of two common shredder species at two different temperatures ... 118
- 6.1. Introduction ... 118
- 6.2. Methods ... 123
    - 6.2.1. Leaf discs and fungus culture preparations ... 123
    - 6.2.2. Animal collection and maintenance ... 123
    - 6.2.3. Experimental system ... 124
    - 6.2.4. Data analysis ... 126
- 6.3. Results ... 127
    - 6.3.1. Survival rate ... 127
    - 6.3.2. Per capita leaf mass loss ... 127
    - 6.3.3. Body mass ... 131
    - 6.3.4. Feeding rate ... 132
- 6.4. Discussion ... 134
- 6.5. Conclusion ... 136

Chapter 7: General discussion ... 138
- 7.1. Main findings ... 139
    - 7.1.1. Aquatic macroinvertebrate communities in agricultural ponds and streams ... 139
    - 7.1.2. Leaf litter decomposition in agricultural ponds and streams ... 140
    - 7.1.3. Freshwater algal communities in agricultural ponds and streams ... 141
    - 7.1.4. Intraspecific interactions and the feeding rate of key shredder species at two different temperatures ... 142
- 7.2. Synthesis ... 143
- 7.3. Implications for ecosystem services and sustainable intensification of agriculture ... 149
- 7.4. Conclusion ... 152

References ... 153

Appendix A ... 175

Appendix B ... 180

Chapter 1: General introduction

1.1. Introduction

By 2050, it is estimated that the global human population will increase to over 9 billion and consequently the global demands for food, water, and energy will increase by at least 50% (Godfray *et al.*, 2010). For example, it is estimated that global demand for food will increase by 70% in 2050 (Foresight, 2011) and the need to feed 9 billion people has been referred to as the global food security challenge (Godfray *et al.*, 2010). Meeting this food security challenge is not straightforward with potential options including: increase in the area of agricultural land, increase in the productivity of existing agricultural land, reduced food waste, improved food distribution and changes in diet (Godfray *et al.*, 2010; Foley *et al.*, 2011; Godfray & Garnett, 2014). The following discussion is focussed on increasing food production.

Agricultural land (croplands and pastures) has been estimated to cover approximately 38% of the global land area: 12% of the total land is currently in use for agricultural crops and 26% of the total land is covered with pastures (Foley *et al.*, 2011). The remaining 37% of potentially cultivatable land is in protected areas or is high biodiversity habitat including forests and wetlands (FAO, 2011). Converting this land to agriculture will therefore have major environmental consequences such as habitat degradation, biodiversity loss, greenhouse gas emissions and water pollution (Godfray & Garnett, 2014). So, global food production will need to increase without substantial use of more land (Foresight, 2011; Godfray & Garnett, 2014).

Global crop productivity has been increased in last recent decades as a result of advanced agricultural technologies, the “Green Revolution” (Evenson & Gollin, 2003; Foley *et al.*, 2005). These technologies included chemical fertilisers that supplied crops with extra nutrients as well as synthetic pesticides that controlled weeds and prevented diseases, and therefore, together resulted in increased yields (Matson *et al.*, 1997). In addition, high-yield crops, which were specifically designed to be more resistant and to produce higher yields, were developed and introduced as well as modern irrigation and multiple cropping, which allowed two or more crops to be grown in the same year (Pinstrup-Andersen & Hazell, 1985; Matson *et al.*, 1997; Evenson & Gollin, 2003). As a result of all these new farming techniques and advances, agricultural production increased remarkably and made it possible to provide food for the growing human population by growing more crops on the same area of land and reducing production costs and food prices (Pinstrup-Andersen & Hazell, 1985; Tilman *et al.*, 2002; Foley *et al.*, 2005). For example, global cereal production more than doubled between the years 1960 and 2000 (Tilman *et al.*, 2002). Although the reliance on pesticides and fertilisers in agriculture contributed to a remarkable increase in productivity, the extensive use of these chemicals resulted in major environmental consequences (Pimentel, 1996; Stoate *et al.*, 2001; Tilman *et al.*, 2001; Foley *et al.*, 2005).

The increased food production will continue to adversely impact the environment, undermine the global capacity for food supply and contribute to climate change and biodiversity loss (Steinfeld *et al.*, 2006; Foresight, 2011). The increased food production will cause several environmental problems including soil degradation (e.g. erosion, salination, reduced fertility), increased water extraction rates for irrigation, high reliance on fossil fuel as an energy source for pesticide and fertilizer synthesis (Foresight, 2011). Furthermore, the production of food will release many pollutants (e.g. nutrients, greenhouses gases, pesticides) which accumulate in the environment (Tilman *et al.*, 2001; Haygarth & Jarvis, 2002).

The need to minimize the adverse impacts of agriculture on the environment will become imperative. This thesis focuses on freshwater habitats because they are among the more vulnerable ecosystems that are being degraded by agricultural land use (Foley *et al.*, 2005; Dudgeon *et al.*, 2006). Increasing agricultural activities have affected water quality of freshwater bodies through nutrient release (resulting from fertilizer use and soil management) and pesticide use (Holden *et al.*, 2015). For example, about 60% of the total loading of nitrogen and phosphorus in the EU is caused by agriculture (Stoate *et al.*, 2009). Increased chemicals and sedimentation from agricultural practices in freshwater can lead to biodiversity loss and changes in biological communities and in their functioning in ecosystems (Della Bella & Mancini, 2009; Piscart *et al.*, 2011; Johnson & Angeler, 2014; Voß *et al.*, 2015).

Understanding the impacts of agricultural land use on freshwater habitats and how organisms respond to these impacts is an important element for agricultural farming improvement and environmental protection. This thesis investigates how different types of agricultural land use, including arable fields and improved grasslands, affect aquatic community structure and function and the consequences on ecosystem service delivery. This chapter discusses the impacts and benefits of agricultural intensification and illustrates the role of biodiversity in the functioning of ecosystems and the delivery of ecosystem services. There is then a description of the UK agricultural landscape before the study aims and objectives are explained.

1.2. Intensification of agriculture

More than 50% of the world's human population live in cities (WHO, 2013; Godfray & Garnett, 2014) and urbanisation will increase with increasing population size (United Nations, 2012). This will result in more land being used for housing and infrastructure and hence less land being available for food production (Ewert *et al.*, 2005; UK National Ecosystem Assessment, 2011). For example, the UK population increased from 50 million in 1950 to over 61 million in 2008, and it will grow to about 72 million by 2033, with an associated increase in demand for goods from ecosystems such as food, water and energy (ONS, 2009; UK National Ecosystem Assessment, 2011).

Agriculture is essential for the provision of food and the maintenance of human well-being. More than 70% of the UK land area is used for agricultural land, including arable and horticultural crops, temporary and permanent grassland, common rough grazing, uncropped arable land and land used for outdoor pigs (Defra, 2013). Agricultural productivity in the UK increased by 40% between 1940 and 1980 and milk production doubled from 1960 to 2009 (UK National Ecosystem Assessment, 2011). This increased food production is a result of agricultural intensification and the increased use of pesticides and fertilizers (Tilman *et al.*, 2001; Foley *et al.*, 2011). Intensively managed agricultural ecosystems are the main source of food production in Europe and cover about 45% of the European territory (EASAC, 2009).

The cultivation and management of land for both crop and livestock production modify habitats and result in changes in species composition and biodiversity loss (Dudgeon *et al.*, 2006; Steinfeld *et al.*, 2006; UK National Ecosystem Assessment, 2011). The use of fertilizers and pesticides to increase agricultural production may result in a decline in the quality and quantity of aquatic and terrestrial habitats (Tilman *et al.*, 2001; Dudgeon *et al.*, 2006; Norris, 2008; Foresight, 2011; UK National Ecosystem Assessment, 2011). The more intensive the agricultural system, the greater the degradation of habitats and hence the greater the loss of biodiversity (Tilman *et al.*, 2001).

Intensification of agriculture is usually associated with removal of natural vegetation and increased chemical inputs, as well as ploughing and increased livestock densities (Allan, 2004; Steinfeld *et al.*, 2006). These changes have significant impacts on freshwater ecosystems including water quality and aquatic communities (Allan, 2004; Dudgeon *et al.*, 2006). Changes in land use that involve the clearing of riparian trees are resulted in increased light availability and water temperature and reductions in organic matter inputs to water bodies (Gregory *et al.*, 1991; Campbell *et al.*, 1992) and these will have consequences for nutrient cycling and benthic algal biomass (Allan, 2004). Further, cultivated land is an important source of sediment inputs to freshwater habitats (Haygarth & Jarvis, 2002). In the UK, it has been estimated that about 75% of sediments polluting freshwater has been derived from agricultural land (Holden *et al.*, 2015). Increased sediment inputs increase water turbidity and modify benthic habitats (Haygarth & Jarvis, 2002) and this has the consequence of changes in food webs including a decrease in primary production and a depletion of food availability to other aquatic organisms (Henley *et al.*, 2000).

Agricultural intensification is generally associated with increased inputs of pesticides which may have negative effects on aquatic biodiversity (Tilman *et al.*, 2001; Haygarth & Jarvis, 2002; Dudgeon *et al.*, 2006) and ecosystems in agricultural landscapes (Dale & Polasky, 2007; Persson *et al.*, 2010; Galic *et al.*, 2012). For example, when pesticides are applied to crop fields, they may cause direct effects on aquatic organisms through toxicity and/or decline in physiological processes or indirect effects through changes in the environment (Haygarth & Jarvis, 2002; Schäfer *et al.*, 2011).

The effects of pesticide application on ecosystems depend on many factors, such as the ability of a product to last for a long time or to degrade, and the ability of a compound to move between environmental compartments and to be bioavailable (vanLoon & Duffy, 2005). Although the use of pesticides is regulated in Europe and has declined slightly in recent years (Stoate *et al.*, 2001), pesticides may still reach adjacent ecosystems and several studies have reported pesticide residues in European water bodies (Schriever & Liess, 2007; Sarkar *et al.*, 2008; Vryzas *et al.*, 2011). Pesticides may enter a water body by spray drift, surface runoff, drainage or via infiltration to groundwater (Persson *et al.*, 2010). Whereas it may be possible to use no-spray buffer zones to mitigate the effects of pesticides in spray drift (Maltby & Hills, 2008), adverse effects resulting from other exposure routes are more difficult to mitigate (Schäfer *et al.*, 2011).

Another major impact of agricultural activities on aquatic ecosystems is eutrophication resulting from fertilizer use. Nutrients are essential for all organisms and they play an important role in the functioning of ecosystems (Hatch *et al.*, 2002). However, excess nitrogen and phosphorus entering water bodies can lead to eutrophication and degradation of water quality. Increased nutrient concentrations can result in increased algal production and changes in algal composition (Dodds, 2006) and extreme eutrophication can result in biodiversity loss including loss of fish species (Biggs, 2000). Freshwater ecosystems do have the capacity to regulate nutrient concentrations with more diverse ecosystems being more efficient at removing nutrients and improving water quality than less diverse ecosystems (Cardinale, 2011). However, the increased use of fertilizers in agriculture has exceeded the capacity of many freshwater communities to remove nutrients, leading to increased nutrient concentrations (Dale & Polasky, 2007). It is estimated that around 60% of nitrates and 25% of phosphorous in UK water bodies have been derived from farming (Holden *et al.*, 2015) and nutrient pollution is the main cause of poor water quality in UK ponds (Williams *et al.*, 2010; UK National Ecosystem Assessment, 2011).

1.3. Ecosystem services provided by agricultural habitats

Ecosystem services are the benefits that people obtain from ecosystems (Costanza *et al.*, 1997; Daily, 1997; Millennium Ecosystem Assessment, 2005). These include products such as food, timber and water, regulation of climate, floods, soil erosion and pollination, and other benefits such as aesthetic values, ecotourism and recreation. The ecosystem services concept plays a significant role in clarifying how people rely on, and gain benefits from, ecosystems through the numerous functions and goods they provide (Haslett *et al.*, 2010). Several different classifications of ecosystem services have been proposed (Daily, 1997; De Groot *et al.*, 2002; Millennium Ecosystem Assessment, 2005; Haines-Young & Potschin, 2013). This thesis adopts the Millennium Ecosystem Assessment (2005) classification, which was used in the UK National Ecosystem Assessment (2011) and which divides services into four main categories: provisioning, regulating, supporting and cultural services.

Agricultural ecosystems provide and depend on ecosystem services, such as food production and pest regulation (Swinton *et al.*, 2007; Zhang *et al.*, 2007; Norris *et al.*, 2010; Power, 2010); however, they also receive dis-services that affect other services and reduce productivity (Zhang *et al.*, 2007) (Figure 1.1).

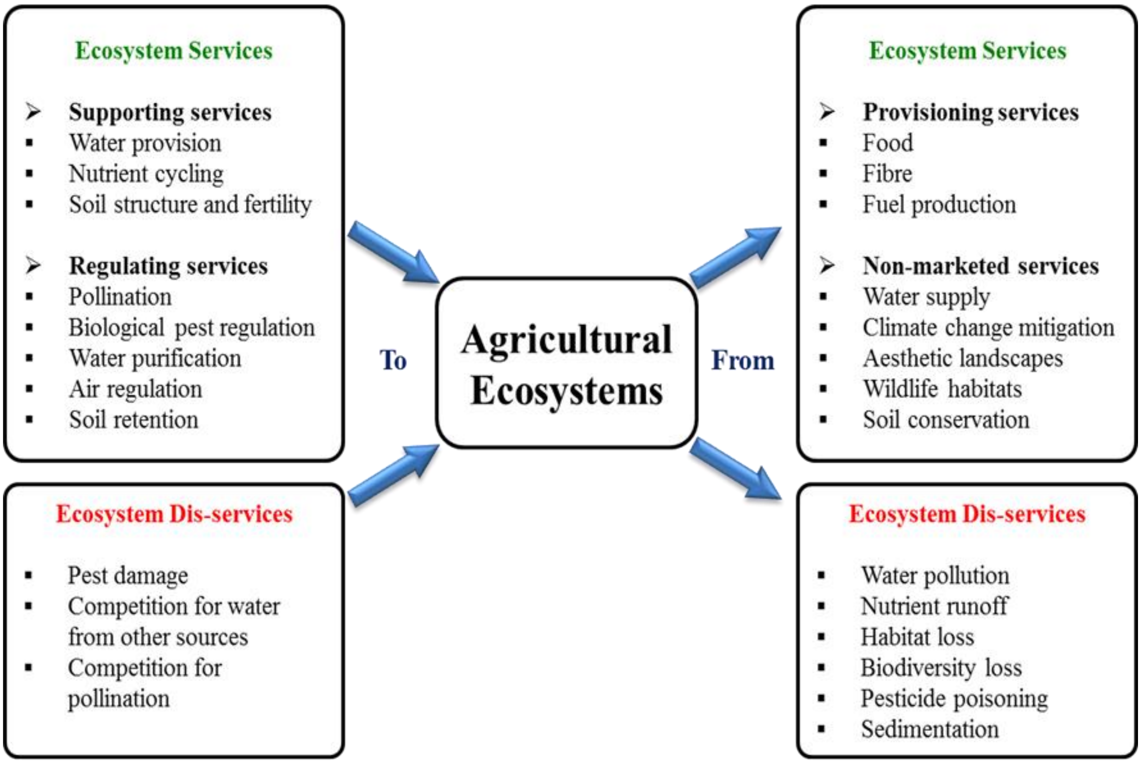

Figure 1.1 Ecosystem services and dis-services required by and provided by agricultural ecosystems, redrawn from Zhang *et al.* (2007).

Although agricultural landscapes are managed mainly for provisioning services such as food production, they also provide other regulating services such as pollination and pest regulation, which are not actively managed and are often unvalued, only becoming obvious when they are lost (Swinton *et al.*, 2007). Pollination in agriculture is managed by honeybee populations and it is crucial for agricultural production: about 84% of European crops depend on insect pollination (Power, 2010; Defra, 2011). Further, biological pest control is one of the regulating services which is provided by natural predators in agricultural landscapes, and it is an important service because natural predators, such as birds, flies, spiders, ladybirds and others, control about 99% of crop pests, such as insects, rodents, snails and viruses (Dale & Polasky, 2007). In addition, the historical importance of agricultural landscapes is manifest in providing houses and work for people, and hence cultural heritage and sense of place are the main cultural services of agricultural ecosystems (Harrison *et al.*, 2010). Moreover, agricultural landscapes play a significant role in ecotourism and recreation because of their beauty (Swinton *et al.*, 2007; Harrison *et al.*, 2010; Power, 2010). Agricultural land therefore needs to be managed for multiple services, for example, food production, biodiversity protection, flood regulation and cultural values (Foresight, 2011; Balvanera *et al.*, 2014). However, many ecosystem services are interdependent and therefore changes in one service may negatively affect others (Rodriguez *et al.*, 2006).

Although agriculture provides many ecosystem services, agricultural activities have negative effects on ecosystems and food production may damage the ecosystem services it depends on, including pest regulation, pollination and nutrient cycling (Zhang *et al.*, 2007; Power, 2010; Balvanera *et al.*, 2014; Allan *et al.*, 2015). Understanding and integrating the benefits of agricultural production with the provision of other ecosystem services is an effective way to achieve food production sustainability and environmental protection (Allan *et al.*, 2015).

1.3.1. The role of biodiversity in delivering ecosystem services

It is well known that biodiversity plays an important role in the functioning of ecosystems and underpins the delivery of all ecosystem services (Altieri, 1999; Loreau *et al.*, 2001; Hooper *et al.*, 2005; Balvanera *et al.*, 2006; EASAC, 2009; Godbold *et al.*, 2011; Cardinale *et al.*, 2012). Earlier studies documented the role of biodiversity in ecosystem functioning (Loreau *et al.*, 2001; Balvanera *et al.*, 2006; Cardinale *et al.*, 2006; Naeem *et al.*, 2009); however, the precise relationship between biodiversity and ecosystem function is subject to debate (Loreau *et al.*, 2001; Naeem *et al.*, 2009). Some species play a key role in ecosystem functioning and any addition or loss from a community results in changes in functioning; however, most species that contribute to ecosystem services can be replaced by other species if they are lost (EFSA, 2010). For example, if species are sensitive to a certain stressor and decrease in abundance, they could be compensated by other species that are more resistant and perform a similar function (Vinebrooke *et al.*, 2004). Furthermore, functional diversity is considered more important than taxonomic diversity in the delivery of services in communities with high functional redundancy (Munns *et al.*, 2009). However, if there is a large decrease in the number of species, functional diversity may be exhausted (Schäfer *et al.*, 2007). Moreover, species richness within functional groups is important in that it enables ecosystems to deal with negative impacts from multiple stressors (Yachi & Loreau, 1999). It should be kept in mind that species usually provide more than one service and may differ in their functional importance in different services (EFSA, 2010).

Ecosystem functions drive the delivery of many ecosystem services. However, the mechanisms by which biodiversity influences the delivery of ecosystem services are relatively poorly understood. Cardinale *et al.* (2012) reviewed two decades of studies investigating how biodiversity affects ecosystem services (mainly provisioning and regulating services). They found that the correlation between ecosystem service delivery and biodiversity was consistent with expectations for 59% of the services included in the review; counter to expectations for 14% of services, and for the remaining for 27% of the services, the evidence for the impact of biodiversity is mixed. Similarly, Balvanera *et al.* (2014) reviewed recent observations, experiments and syntheses on the impacts of biodiversity change, focusing on species richness, on selected ecosystem services: timber, forage, climate regulation, fisheries, water quality and agricultural pest control. They identified the uncertainties that impede the understanding of processes that link biodiversity change to ecosystem services; these uncertainties included limited data and a mismatch between the measured variables and the final service that is related to stakeholders. They concluded that whereas some of the findings support the links between species richness and ecosystem services, uncertainties associated with many studies limited the ability to draw clear conclusions.

Harrison *et al.* (2014) analysed the link between different attributes of biodiversity and 11 ecosystem services. They reported that five biodiversity attributes (species abundance, species richness, species size/weight, community/habitat area and community/habitat structure) were cited as being important for at least one service in over 50% of papers; three attributes (community/habitat age, aboveground and belowground biomass) were cited as being important for at least one service in 25 to 50% of papers; and the functional group attributes were cited as being important for pollination and pest regulation in 14 and 22% of papers. In managed grassland ecosystems, Allan *et al.* (2015) provided evidence of a strong trade-off between the delivery of cultural and provisioning services in agricultural ecosystems. They found strong overall effects of land use intensification on multiple ecosystem services delivery and concluded that the direction and strength of these effects depended on the types of services: the effects of land use intensity were positive for provisioning services but negative for cultural services.

A recent study by Pinto *et al.* (2014) attempted to link biodiversity to ecosystem functions, ecosystem services and human well-being in estuarine ecosystems using data from samples collected in 1998, 2005, 2006 and 2007. They found that species composition was an important factor that had strong effects on ecosystem functioning; the ecosystem service provision was determined by the complex relationships between biodiversity and human well-being; and changes in ecosystem service provision probably derive from changes in the community structure and abiotic factors and from the decline or loss of the most abundant species.

Human activities resulting in biodiversity loss have led to alterations in ecosystem functions and changes in associated ecosystem services (Hooper *et al.*, 2005; Cardinale *et al.*, 2012; Allan *et al.*, 2015). Understanding the effects of human disturbance on biodiversity and functioning is therefore essential for the effective management of landscapes for the sustainable delivery of multiple ecosystem services. This thesis contributes to this understanding by investigating the effect of land use on biodiversity and functioning of small freshwater habitats in UK agricultural landscapes.

1.4. Agricultural landscapes in the UK

Enclosed Farmland is one of the largest Broad Habitat categories in the UK, and it has been estimated that in 2007 Enclosed Farmland covered more than 40% of the UK land area (Carey *et al.,* 2008). Enclosed Farmland includes fields used for crop production (arable and horticultural) and grass production (improved grassland), along with networks of hedgerows and field margins and small patches of small woodlands interspersed within them (UK National Ecosystem Assessment, 2011). Enclosed Farmland is mainly managed for food production; most arable land is cultivated to grow food crops (e.g. wheat, barley, oats and oilseed rape) whereas most grassland is managed for livestock grazing (e.g. sheep and cattle) (UK National Ecosystem Assessment, 2011).

Enclosed Farmland is commonly associated with small freshwater habitats such as ponds and streams. Ponds are defined as standing (lentic) water bodies, either permanent or seasonal, with an area between 25 m^2 and 2 ha, and they include natural and man-made water bodies (Williams *et al.*, 2004). Streams are defined as small running (lotic) water bodies, mainly created by natural processes, and defined by the Ordnance Survey (OS) as being less than 8.25 m in width and marked as a blue line on 1:25,000 OS maps (Williams *et al.*, 2004). It is well known that ponds and streams differ in their physical and chemical characteristics as well as in their biological diversity (Williams *et al.*, 2004), and the biological and chemical quality of aquatic habitats is most impacted in areas where surrounding land use is dominated by intensive agriculture or urbanization (Williams *et al.*, 2010).

Small freshwater bodies make an important contribution to the diversity of aquatic plants and invertebrates in agricultural landscapes. Local species richness (alpha diversity) for macrophytes and macroinvertebrates was highest in rivers followed by ponds, streams and ditches whereas regional species richness (gamma diversity) was highest for ponds (Williams *et al.,* 2004). Ponds supported more species and more unique species than other freshwater habitats (Williams *et al.,* 2004).

Freshwater biodiversity plays a key role in the functioning of aquatic ecosystems (Wallace & Webster, 1996; Covich *et al.,* 2004) and underpins many ecosystem services (Suter & Cormier, 2015). Table 1.1 shows the three categories of ecosystem services and associated benefits of aquatic insects in Central Appalachia in the USA listed by Suter and Cormier (2015); however, other aquatic invertebrates can be involved in providing these services and benefits. For example, aquatic invertebrates provide food sources for many aquatic and terrestrial vertebrates such as birds, fishes, amphibians and reptiles (Covich *et al.*, 1999; Suter & Cormier, 2015).

Aquatic invertebrates play an important functional role in freshwater ecosystems, such as leaf litter decomposition (Wallace & Webster, 1996; Graça, 2001). Shredding invertebrate reduce leaf litter to small particles that are consumed by other collectors (Wallace & Webster, 1996), leading to an increase in fish habitats and improvement in stream aesthetics (Suter & Cormier, 2015). Without shredding invertebrates, the leaf litter would accumulate in stream beds, which would reduce habitats for fish eggs and larvae and would decrease dissolved oxygen leading to hypoxic conditions and producing sulphurous odours, and therefore reduce water quality (Suter & Cormier, 2015). In addition, the decomposition of leaf litter in streams releases nutrients that can be taken by other organisms and hence affects nutrient cycling and organic matter production (Webster & Benfield, 1986; Graça, 2001). Grazing invertebrates feed by scraping algae from rocks and organic materials and hence influence the structure and abundance of algal communities (Wallace & Webster, 1996; Covich *et al.*, 1999), which are important for nutrient removal and water purification (Cardinale, 2011). In addition, some invertebrates such as blackfly simuliid larvae remove algae and microbes from water columns and feed on them (Suter & Cormier, 2015).

Aquatic invertebrate communities have been used as a good indicator for assessing the ecological quality of streams and rivers (Rosenberg & Resh, 1993; Suter & Cormier, 2015). They also provide direct benefits to human: for examples, some invertebrates (particularly odonates and mayflies) have aesthetic value and can provide ideas for artists and craftsmen (Suter & Cormier, 2015). Further, aquatic insects can be used as a source of education, exploring and learning activities as well as photography and literary images (Suter & Cormier, 2015).

Understanding the role of aquatic biodiversity in freshwater functioning and the associated benefits is important to protect aquatic communities and to recognise that some species are unique in their functional role and cannot be replaced by other species (Suter & Cormier, 2015). This thesis focuses on benthic macroinvertebrates and algae and their functional role in ponds and streams located in agricultural landscapes. These communities are easily sampled and they have been used as environmental indicators to assess water quality (Rosenberg & Resh, 1993; Kelly & Whitton, 1995).

Table 1.1 The three categories of ecosystem services and associated benefits of aquatic insects as listed by (Suter & Cormier, 2015).

Source of food	Role in ecosystem functioning	Direct human uses
Aquatic insects are food for: • Fish • Amphibians • Birds • Bats • Semi-aquatic mammals	Aquatic insects perform ecosystem functions: • Nutrient retention • Litter decomposition • Cleaning rocks • Stream recovery • Removal of algae and pathogenic microbes • Participation in elemental cycles • Stabilization of stream beds	Aquatic arthropods are used by human in: • Environmental indicators • Fishing • Education • Aesthetics • Art and design • Photography • Literary images and metaphors • Commercial and organisational symbols

1.5. Aim and objectives

Increasing food production to meet the global food security challenge of feeding 9 billion people by 2050 may result in major environmental impacts (Bennett *et al.*, 2015). The structure and functioning of freshwater communities are affected by changes in habitat quality resulting from the management of adjacent land. In agricultural catchments, the two major land uses, arable and pastoral, differ in their management (e.g. ploughing, fertilizer and pesticide inputs) and therefore in their potential effect on freshwater communities. Numerous studies have investigated the impacts of agriculture on freshwater ecosystems, but few studies have compared different types of agricultural practices. In addition, whereas the biodiversity of freshwater habitats within agricultural landscapes has been compared (Williams *et al.*, 2004; Biggs *et al.*, 2007), information on how different water bodies respond to different types of agricultural land use is scarce.

Investigating the changes in community structure and functioning resulting from a specific type of land use provides a better understanding of the potential effects of agriculture on the ecology and ecosystem services provided by freshwater habitats. This understanding, will in turn, enables more effective agricultural management for protecting aquatic ecosystems, increasing food producing and sustaining ecosystem service delivery in agricultural landscapes.

The main aim of this study was to investigate the effects of two agricultural land uses (arable and pastoral) on aquatic communities and on and their ecological functioning in ponds and streams. The sites used in the current study were part of the Water Friendly Farming (WFF) project (Biggs *et al.*, 2014) based at Loddington in Leicestershire, UK (Chapter 2).

This study aimed to answer the following questions: (1) What impacts do different types of agricultural land use have on macroinvertebrate communities in ponds and streams? (2) How is leaf litter decomposition in ponds and streams affected by agricultural land use? (3) How do algal communities in ponds and streams located in agricultural catchments respond to different types of land use? (4) How do intraspecific interactions affect leaf decomposition by freshwater shredding invertebrates at two different temperatures? These questions were addressed via the following objectives:

1. To investigate the impact of agricultural land use (arable and pastoral) on aquatic macroinvertebrate communities in ponds and streams (Chapter 3). This was achieved by sorting and identifying macroinvertebrate samples collected from 12 ponds and 12 streams. The structure, composition and functional role of invertebrates were analysed and then compared between arable and pastoral sites.

2. To explore how different types of agricultural land use influence leaf litter decomposition in ponds and streams (Chapter 4). This was addressed by quantifying leaf litter processing using coarse and fine leaf bags deployed in 12 ponds and 12 streams. Both total and microbial leaf decomposition were assessed and then compared between arable and pastoral sites. In addition, in situ feeding rate of the shredding invertebrate (*Gammarus pulex*) was measured in 12 stream sites and then compared between arable and pastoral sites.

3. To compare algal communities in ponds and streams in different agricultural land uses (Chapter 5). This was achieved by measuring chlorophyll *a* concentration (i.e. total algal biomass) and identifying diatom species in 12 ponds and 12 streams. Algal biomass and diatom community structure were analysed and then compared between arable and pastoral sites.

4. To explore the relative importance of biotic interactions (intraspecific) between key shredder species and the subsequent impacts on leaf decomposition at two different temperatures (Chapter 6). This was achieved by conducting two feeding experiments using three abundance treatments (individual, group of 5 and group of 15) and two different temperatures (high 21°C and low 7°C) with two shredding invertebrate species (*G. pulex* and *Asellus aquaticus*). The survival rates of study species were quantified and per capita feeding rate and leaf mass loss were analysed.

A synthesis of the main research findings is provided in Chapter 7, which then discusses how they contribute to understanding the effects of different types of agricultural land use on freshwater communities and their ecological functioning. Implications for ecosystem service delivery and the sustainable intensification of agriculture are discussed.

Chapter 2: Study site descriptions

The sites used in this study were part of the Water Friendly Farming (WFF) project jointly run by the Game & Wildlife Conservation Trust, Freshwater Habitats Trust and Syngenta (Biggs *et al.*, 2014). The aim of the WFF project is to investigate the effectiveness of rural land mitigation measures to reduce the impacts of agricultural land use on freshwater habitats. These impacts include increased levels of agricultural pollutants (e.g. phosphorus, nitrogen and pesticides), sedimentation and physical modification of freshwater bodies. The WFF project is focussed on three small agricultural catchments in Leicestershire, UK: Barkby Brook, Eye Brook and Stonton Brook catchments (Figure 2.1). These catchments are typical of the agricultural system in the region and consist of a mixture of arable and grassland farming. The WFF project studied streams, ditches and ponds, and 180 sites (60 streams, 60 ditches, 60 ponds) were selected at the start of the project in 2010.

Agricultural landscapes in the UK have been divided into twelve classes based on soil properties, hydrogeology, topography and cropping (Brown *et al.*, 2006). Two of the 12 landscape classes were represented in the study area: Land Class 4 (eutrophic tills) and Land Class 6 (pre-Quaternary clay) (Biggs *et al.*, 2014). Soils in the study area are heavy to medium clay with some sandy outcrops, and are mainly poorly draining (Biggs *et al.*, 2014). They are described in the National Soil Resources Institute NSRI Soilscapes dataset as mainly slowly permeable, seasonally wet, slightly acid but base-rich loamy and clayey soils (Farewell *et al.*, 2011).

Figure 2.1 A map shows the location of the study catchments of the WFF project in Leicestershire, UK. The shaded red area shows the boundary of Barkby Brook, Eye Brook and Stonton Brook catchments. Scale 1:1000000, Source: EDINA Digimap Ordnance Survey Service ‘Roam’ application, <http://digimap.edina.ac.uk> , Downloaded: 28/ 10/ 2015.

The WFF project has been sampling sites in all three catchments annually since 2010. Each year 60 pond, 60 stream and 60 ditch sites are sampled for wetland plants (in autumn since 2010) and aquatic invertebrates (in spring since 2011). In addition, fish were surveyed in 2012 and 2013 and diatom samples were collected in 2013 (in spring, summer, autumn). Water samples have been collected each spring since 2011 for measurement of nutrient concentrations and water flows have been measured at each catchment outfall since January 2012 (Biggs *et al.*, 2014).

2.1. Site selection

The current study was based in two of the three WFF study catchments, Eye Brook and Stonton Brook. Land use in Eye Brook catchment was 45% arable, 42% improved grassland and 13% other land use categories, whereas in Stonton Brook catchment it was 44% arable, 41% improved grassland and 15% other land use categories (Biggs *et al.*, 2014) (Figure 2.2). Arable fields were mainly planted with winter wheat and oilseed rape, with some fields planted with beans and oats, and improved grassland was used for sheep and cattle grazing.

Twenty-four WFF sites were selected: three arable and three pastoral ponds per catchment plus three arable and three pastoral streams per catchment. Sites were initially selected from a long list of sites using land use maps provided by the WFF project, and then selected sites were confirmed during field visits. A handheld GPS navigator (GARMIN, Oregon ^{TM} 300) was used to collect a point data file for each site during field visits. Latitude, longitude and altitude for each site were determined using the GPS navigator, whereas data on estimated shade percentage, pond areas and stream channel widths were provided by the WFF project. National grid reference, altitude, estimated shade (%) and pond area or stream channel width are presented in Table 2.1.

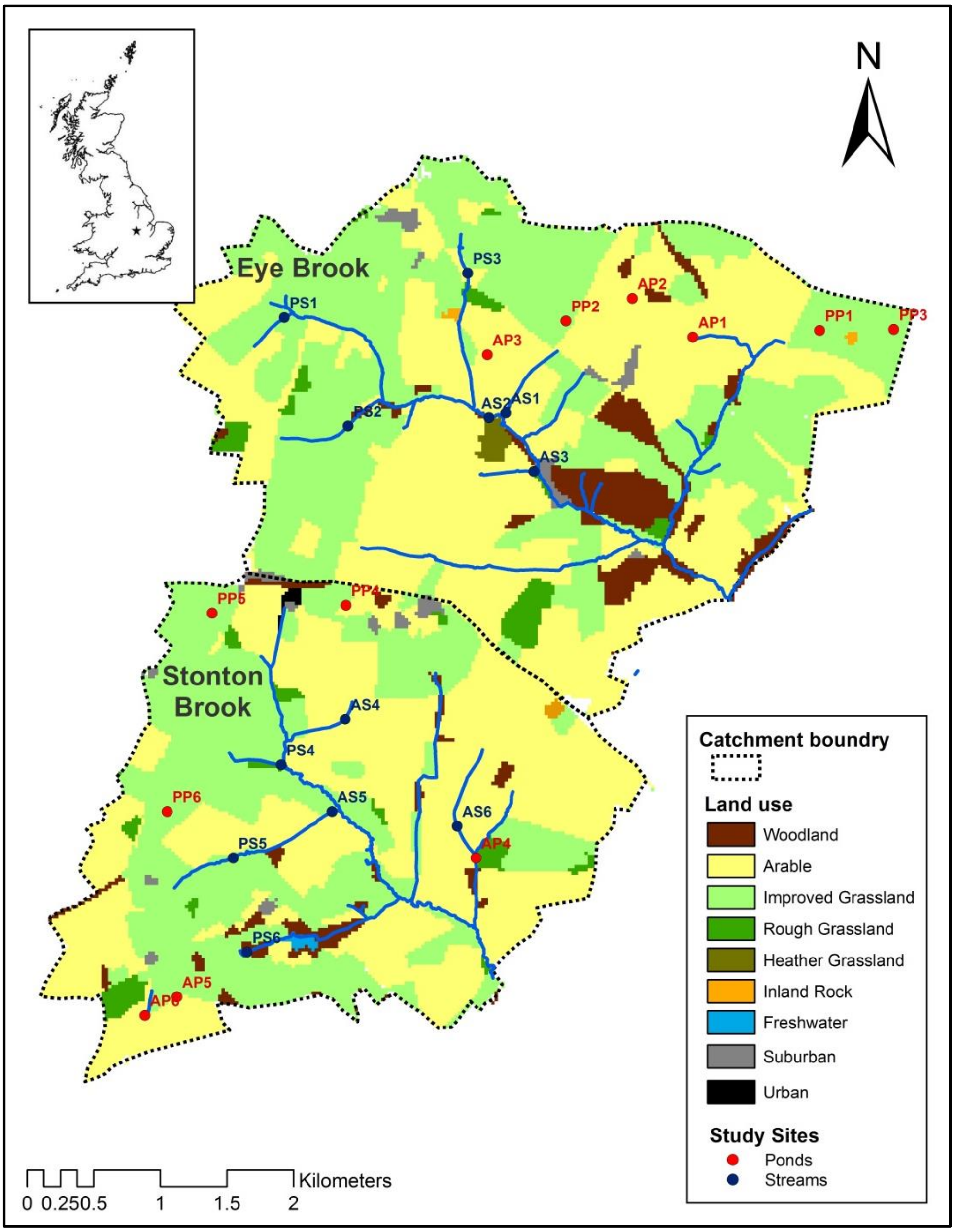

Figure 2.2 Land Cover Map 2007 for the study catchments: Eye Brook and Stonton Brook in Leicestershire, UK. Six ponds (red symbols) and six streams (blue symbols) were studies in each catchment. AP: arable pond, PP: pastoral pond, AS: arable stream and PS: pastoral stream. Source: Great Britain 25m [TIFF geospatial data], Scale 1:250000, Tiles: GB, Updated: 2007, CEH, Using: EDINA Digimap Ordnance Survey Service, <http://digimap.edina.ac.uk>, Downloaded: 16/07/2014

Table 2.1 National grid reference, altitude and area or channel width and estimation shade of ponds and stream sites.

Land use and water body type	Catchment	Site	National Grid Reference	Altitude (m)	Area (m^2)	Channel Width (cm)	Shade (%)
Arable Ponds	Eye	AP1	SK 763 047	171.46	210	-	25
	Eye	AP2	SK 759 050	195.57	60	-	75
	Eye	AP3	SK 748 046	174.13	450	-	0
	Stonton	AP4	SK 747 008	143.38	2000	-	25
	Stonton	AP5	SP 725 997	176.29	220	-	75
	Stonton	AP6	SP 722 996	164.93	550	-	50
Pastoral Ponds	Eye	PP1	SK 773 047	178.88	160	-	0
	Eye	PP2	SK 754 048	187.79	75	-	0
	Eye	PP3	SK 778 047	188.77	110	-	75
	Stonton	PP4	SK 738 026	194.47	375	-	25
	Stonton	PP5	SK 727 026	208.25	85	-	25
	Stonton	PP6	SK 724 011	193	75	-	0
Arable Streams	Eye	AS1	SK 749 041	155.8	-	100	100
	Eye	AS2	SK 748 041	156.67	-	180	75
	Eye	AS3	SK 750 037	149.45	-	110	0
	Stonton	AS4	SK 737 018	174.23	-	120	75
	Stonton	AS5	SK 736 011	139.09	-	205	75
	Stonton	AS6	SK 746 010	143.89	-	100	100
Pastoral Streams	Eye	PS1	SK 732 048	50.87	-	100	50
	Eye	PS2	SK 737 040	22.86	-	125	0
	Eye	PS3	SK 746 052	35.59	-	120	75
	Stonton	PS4	SK 732 015	159.88	-	110	50
	Stonton	PS5	SK 729 008	159.6	-	70	0
	Stonton	PS6	SK 730 001	156.85	-	70	100

Land use for the study catchments was assessed using the ESRI ArcGIS 10.1 software. Three datasets were used: (1) Land Cover Map (LCM) 2007 layer downloaded from the EDINA Digimap Ordnance Survey; (2) the shape geospatial data ”Open Rivers” downloaded from the EDINA Digimap Ordnance Survey and (3) the polygon shape data files for the catchment boundaries provided by WFF project. The locations of the study sites within land use are shown in Figure 2.2.

Assessment of land use using LCM 2007 confirmed the initial classification of study sites as either ‘arable’ or ‘pastoral’ except for one of the pastoral streams (PS3 in Eye Brook). According to LCM 2007, PS3 is located within an arable land use area (Figure 2.2); however, field visits and analysis of Google Earth images from 2000, 2006 and 2011 confirmed that the site has been within a grassland area since at least 2000 (Figure 2.3). Images of some ponds and streams are presented in Figures 2.4 and 2.5, respectively.

Figure 2.3 Google Earth images for the location of pastoral stream **PS3** in different years: a) 2000, b) 2006 and c) 2011.

Figure 2.4 Example photographs of pond sites: **AP3** and **AP4** are arable ponds; **PP1** and **PP5** are pastoral ponds.

Figure 2.5 Example photographs of stream sites: **AS3** and **AS5** are arable streams; **PS2** and **PS5** are pastoral streams.

2.2. Site-specific land use, elevation and flow patterns

All study sites were located within either arable or pastoral land use but some sites were located near the boundary of two land use types and therefore may have been influenced by both land uses. The estimation of land use with 100 radius was previously used (Brown *et al.*, 2006). Land use within a 100 m radius circular area of each pond or within a semi-circle area with a 100 m radius upstream of each stream site, was determined using the ESRI ArcGIS 10.1 software with LCM 2007 downloaded from the EDINA Digimap Ordnance Survey. Sites with ≥ 75% arable fields in the surrounding area were classified as ‘arable’, sites with ≥ 75% improved grassland were classified as ‘pastoral’ and sites with < 75% arable farmland or improved grassland were classified as ‘mixed’ (Figure 2.6 and 2.7) .

Eleven sites were surrounded by a single land use, four sites (AP4, AP5, PP4 and AS5) were classified as mixed and the remainder had a least 75% of the surrounding land use within a single land use category (Table 2.2). Sites surrounded by a single land use type were not analysed further. For the other sites, water flow direction was assessed to determine which land use would be the primary source of runoff and hence contamination.

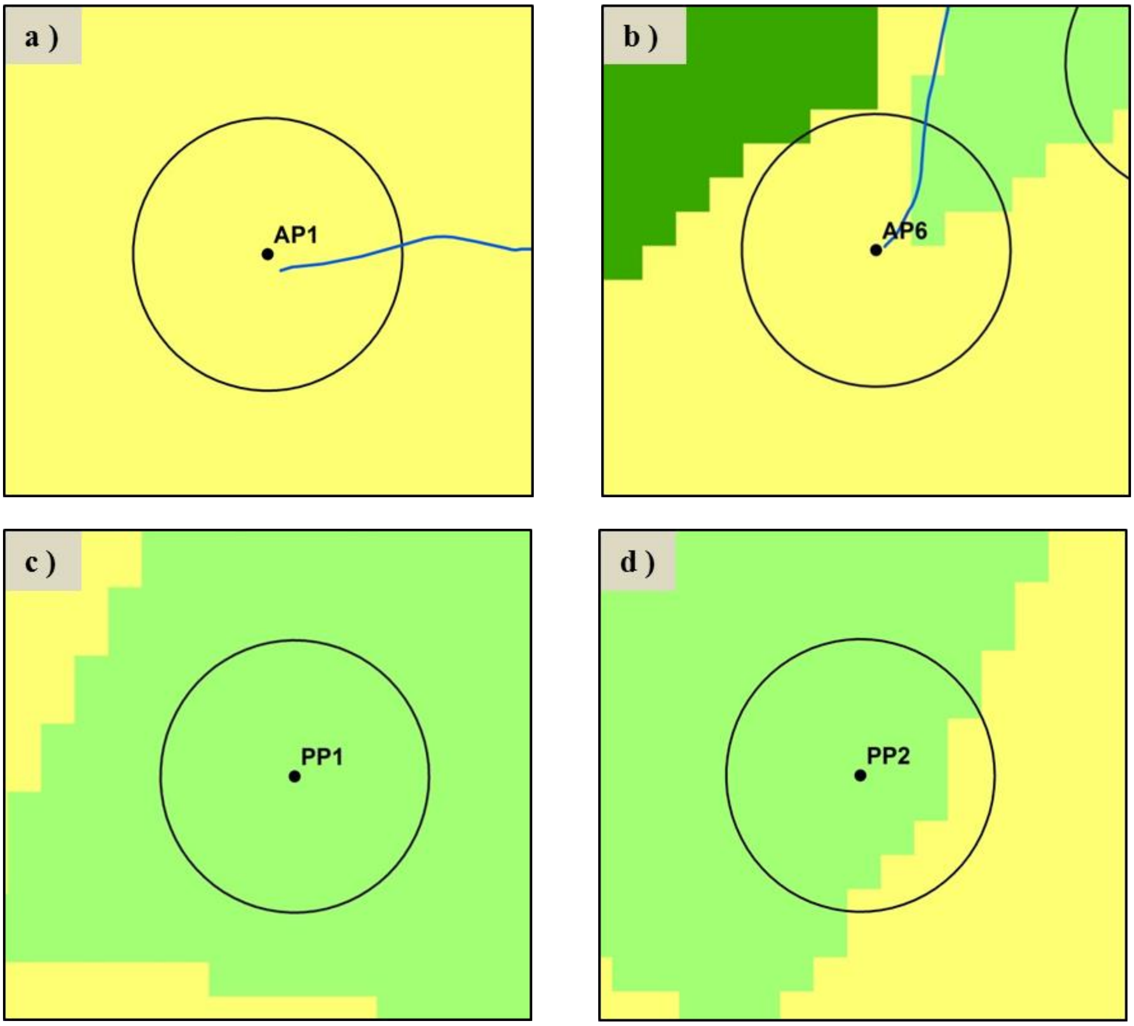

Figure 2.6 Examples of site-specific land use estimations in arable ponds (a, b) and pastoral ponds (c, d). Colour codes for land use are described in Figure 2.2.

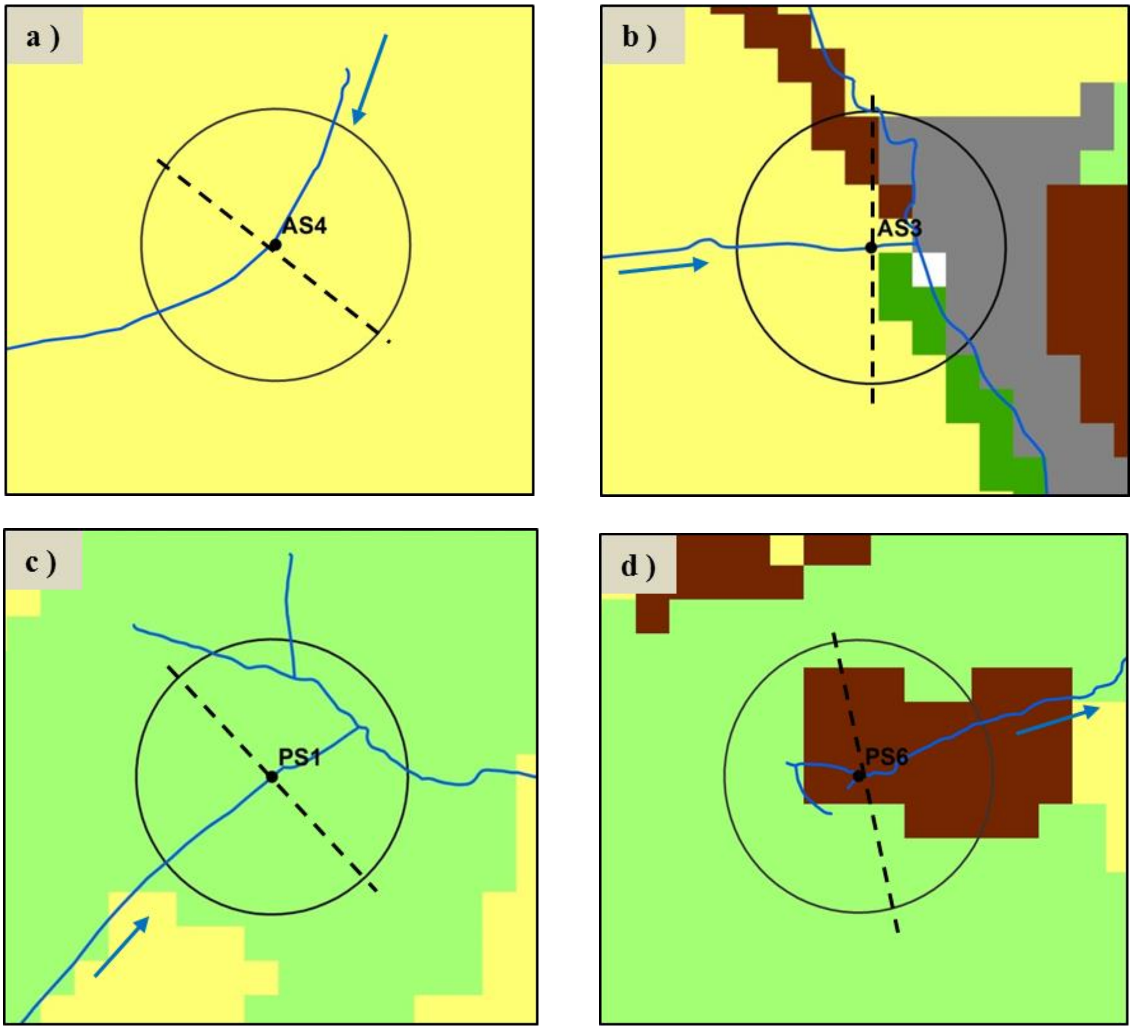

Figure 2.7 Examples of site-specific land use estimations in arable streams (a, b) and pastoral streams (c, d). Blue arrows represent the upstream flow direction. Colour codes for land use are described in Figure 2.2.

Table 2.2 Estimations of surrounding land use and source of water flow to study sites with resulting land use classifications. The pastoral stream PS3 was excluded from land use estimations due to inaccurate LCM 2007 data.

General land use and water body type	**Catchment**	**Site**	**Surrounding land use**			**Classification based on surrounding land use**	**Classification based on water flow source**	**Final land use classification**
			Arable (%)	Pastoral (%)	Other (%)			
Arable Ponds	Eye	AP1	100	0	0	Arable	-	Arable
	Eye	AP2	100	0	0	Arable	-	Arable
	Eye	AP3	96	4	0	Arable	100% Arable	Arable
	Stonton	AP4	60	0	40	Mixed	75% Arable	Arable
	Stonton	AP5	46	54	0	Mixed	40% Arable	Mixed
	Stonton	AP6	88	12	0	Arable	100% Arable	Arable
Pastoral Ponds	Eye	PP1	0	100	0	Pastoral	-	Pastoral
	Eye	PP2	18	82	0	Pastoral	100% Pastoral	Pastoral
	Eye	PP3	0	100	0	Pastoral	-	Pastoral
	Stonton	PP4	38	62	0	Mixed	100% Pastoral	Pastoral
	Stonton	PP5	0	100	0	Pastoral	-	Pastoral
	Stonton	PP6	0	100	0	Pastoral	-	Pastoral
Arable Streams	Eye	AS1	100	0	0	Arable	-	Arable
	Eye	AS2	80	0	20	Arable	80% Arable	Arable
	Eye	AS3	92	0	8	Arable	100% Arable	Arable
	Stonton	AS4	100	0	0	Arable	-	Arable
	Stonton	AS5	50	50	0	Mixed	60% Arable	Mixed
	Stonton	AS6	100	0	0	Arable	-	Arable
Pastoral Streams	Eye	PS1	0	100	0	Pastoral	-	Pastoral
	Eye	PS2	0	100	0	Pastoral	-	Pastoral
	Stonton	PS4	0	88	12	Pastoral	90% Pastoral	Pastoral
	Stonton	PS5	25	75	0	Pastoral	100% Pastoral	Pastoral
	Stonton	PS6	0	80	20	Pastoral	100% Pastoral	Pastoral

Water flow direction is determined by topography and therefore an elevation model and water flow map for the study area were generated using the ESRI ArcGIS 10.1 software and three datasets: (1) Digital Terrain Model downloaded from the EDINA Digimap Ordnance Survey “PANORAMA DTM”; (2) the shape geospatial data ”Open Rivers” downloaded from the EDINA Digimap Ordnance Survey and (3) the polygon shape data files for the catchment boundaries provided by WFF project. The catchment elevation model and water flow direction map are shown in Figure 2.8. Flow direction for each site was determined for the same area used to determine surrounding land use area (Figures 2.9 and 2.10).

Among sites which had at least 75% of the surrounding land use within single land use category, six sites were classified as 100% of that land use (Table 2.2). For others (AS2 and PS4), there was a possible water flow (10 - 20%) from other land use categories (e.g. woodland or rough grassland) (Table 2.2). The stream site (AS2) was classified as ‘80% arable’ based on water flow source and the stream site (PS4) was classified as ‘90% pastoral’ (Table 2.2, Figure 2.10). Overall, the water flow direction analysis resulted in all these sites (i.e. with > 75%) were classified as that land use (Table 2.2).

For the ‘mixed’ sites, there was a possible water flow to the pond site (AP4) from the surrounding rough grassland (25%) (Figure 2.9a and 2.9b) and it was given a final land use classification as ‘arable’ (Table 2.2). For the mixed pond site (PP4), most of the water flow to the site was from improved grassland field (Figure 2.9e and 2.9f) and therefore it was given a final land use classification as ‘pastoral’ (Table 2.2). However, this analysis resulted in one pond site (AP5) and one stream site (AS5) having a final land use classification ‘mixed’ (Table 2.2, Figure 2.9 and 2.10).

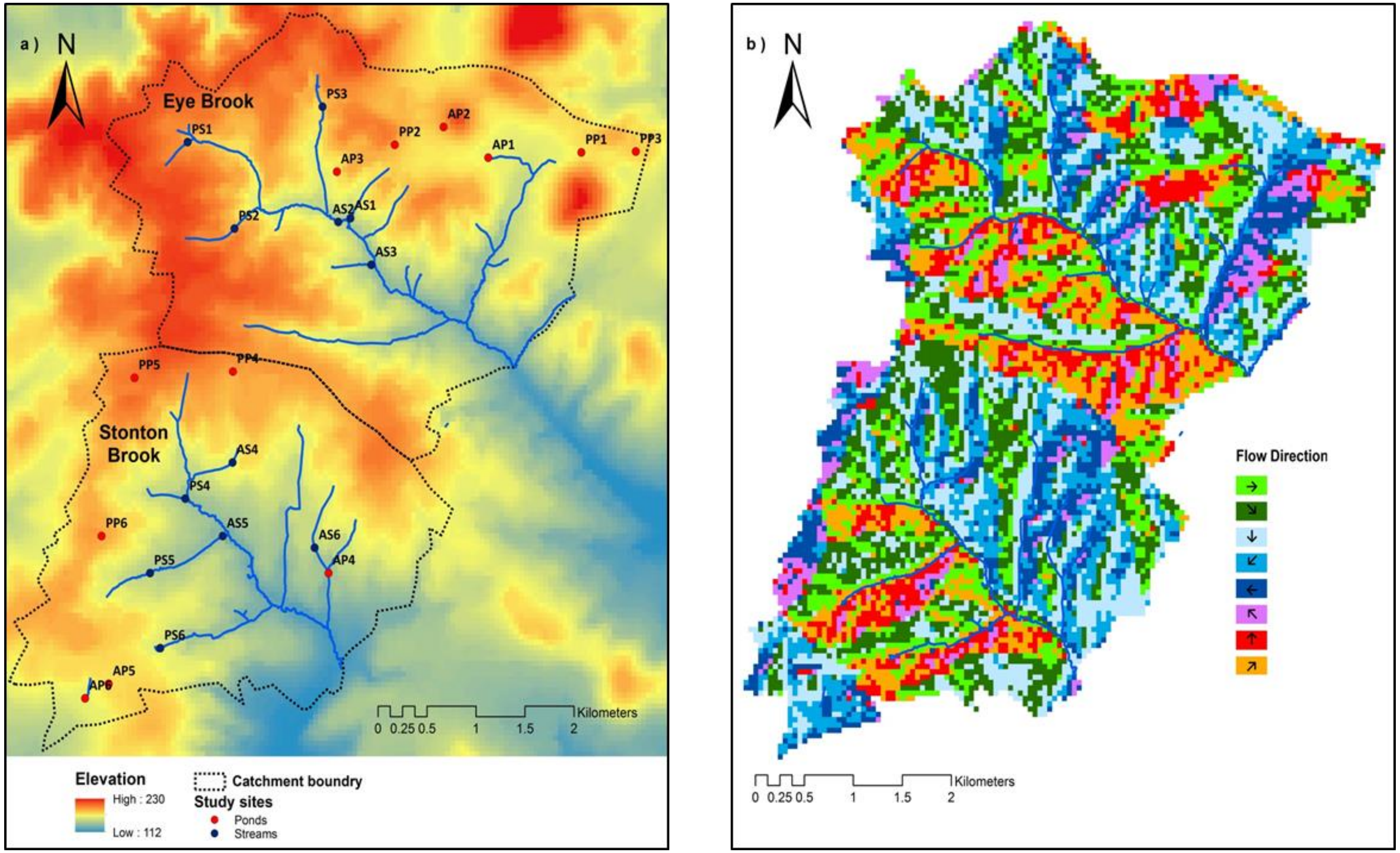

Figure 2.8 a) Digital Terrain Model and b) water flow direction maps of the study catchments (Eye and Stonton) in Leicestershire, UK. Catchment boundaries are delimited by dotted lines and six ponds (red symbols) and six streams (blue symbols) were studies in each catchment and their locations are illustrated in panel a). Colours in panel a) represent elevations with red being high (up to 230 m) and blue being low (down to 112 m) whereas coloured squares in panel b) represent different flow directions as defined in the associated key. Source: PANORAMA DTM [TIFF geospatial data], Scale 1:50000, Tiles: SK6, SK80, SP68, SP88, Updated: 12 June 2006, Ordnance Survey (GB), Using: EDINA Digimap Ordnance Survey Service, <http://digimap.edina.ac.uk>, Downloaded: 20/04/2015.

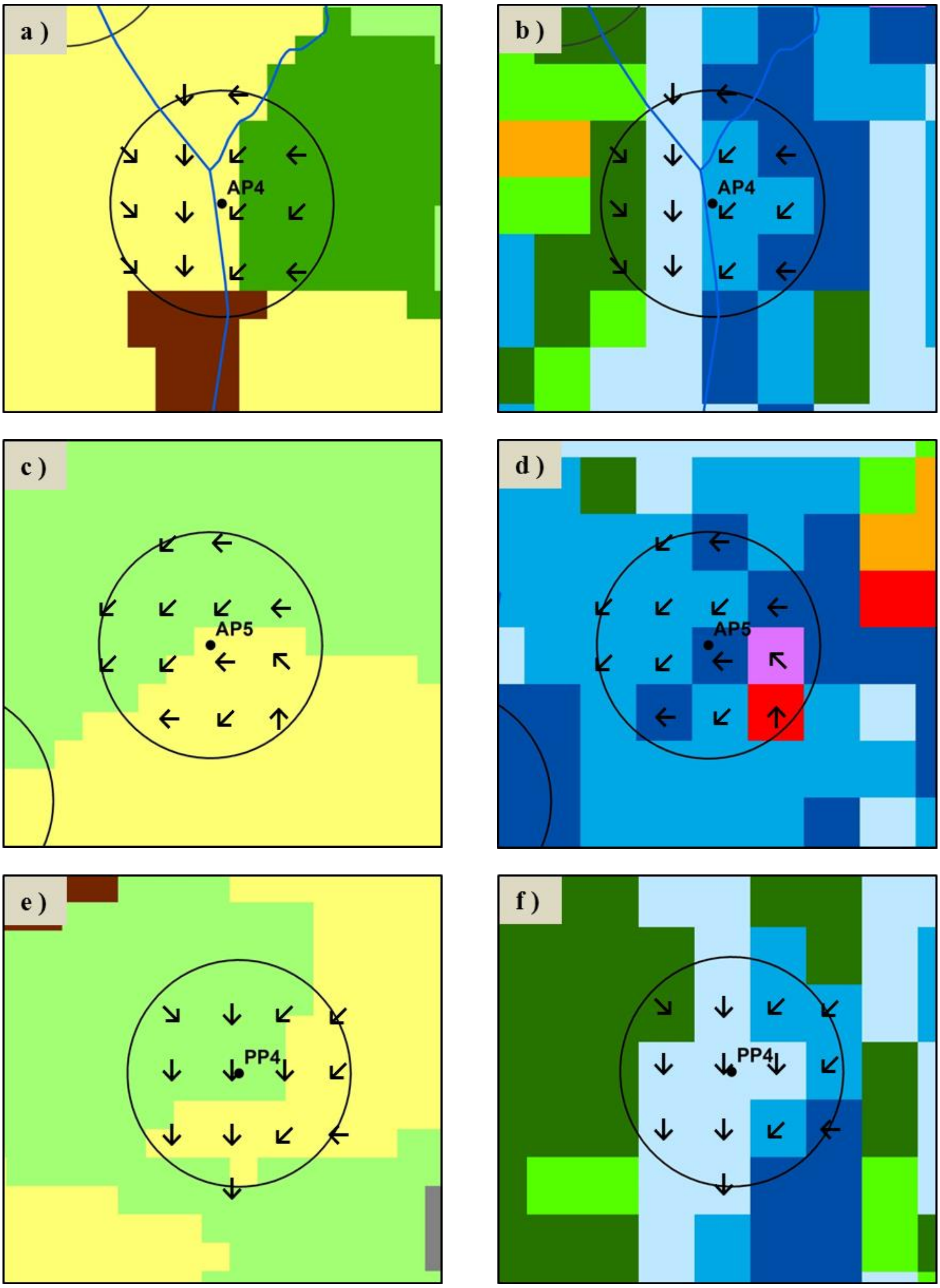

Figure 2.9 Examples of estimation of surrounding land use based on water flow direction to arable ponds **AP4** (a, b) and **AP5** (c, d), and the pastoral pond **PP4** (e, f). Black arrows represent the water flow direction. Colour codes for land use are described in Figure 2.2 and for flow direction are described in Figure 2.8b.

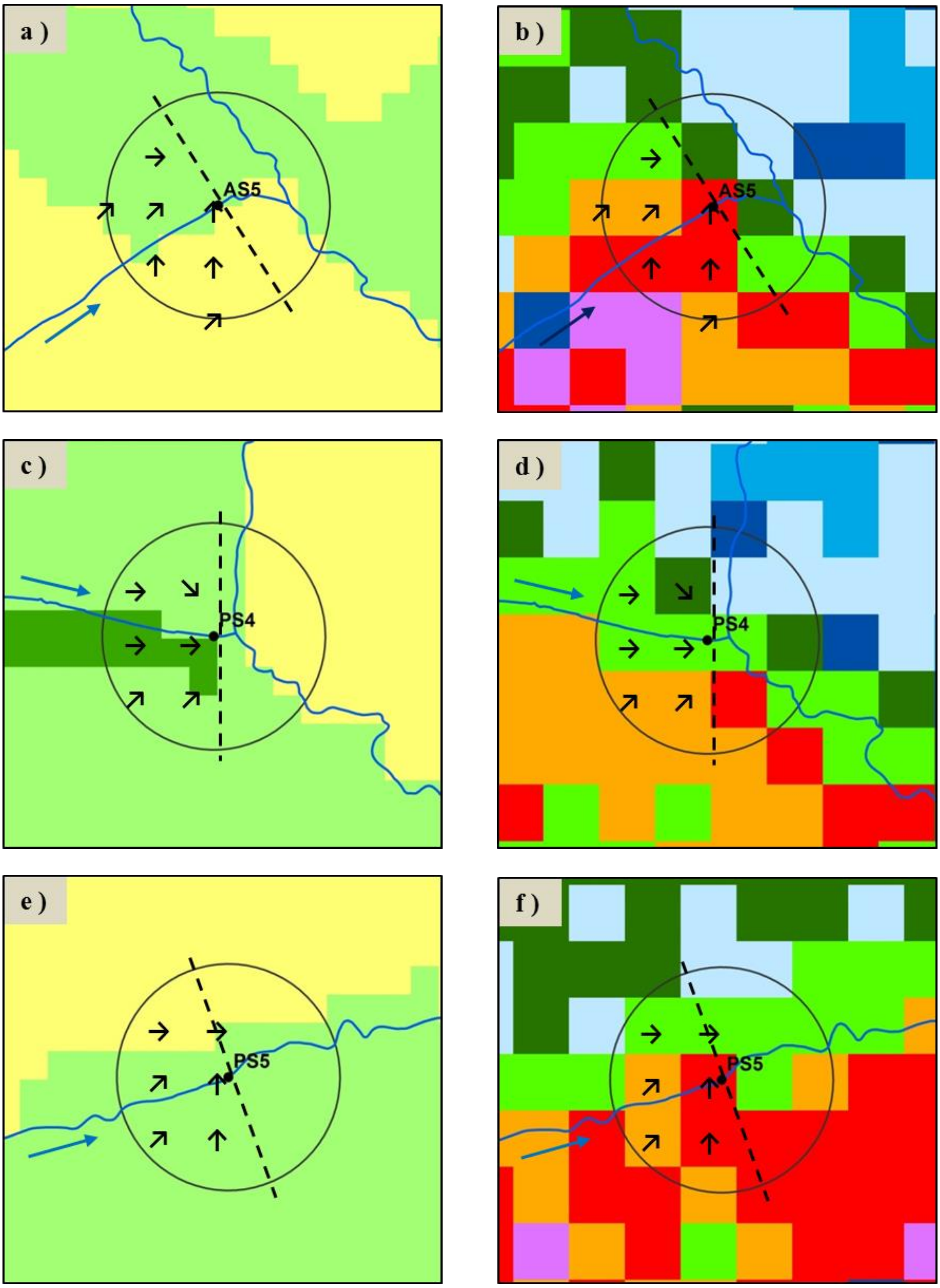

Figure 2.10 Examples of estimation of surrounding land use based on water flow direction to the arable stream **AS5** (a, b), the pastoral streams **PS4** (c, d) and **PS5** (e, f). Black arrows represent the water flow direction and blue arrows represent the upstream flow direction. Colour codes for land use are described in Figure 2.2 and for flow direction are described in Figure 2.8b.

2.3. Physicochemical characteristics of water

The physicochemical characteristics of the study sites were determined by the WFF project in 2011 and 2013 (Table 2.3 and 2.4). In 2014, water quality characteristics were measured as part of the current study and are presented in Chapter 4. The analyses of water samples collected in 2011 were conducted at Oxford Brookes University whereas the analysis of the 2013 samples was performed by the Environment Agency-National Laboratory Service (Biggs *et al.*, 2014).

In 2011, pastoral ponds had a significantly higher concentration of total phosphorus and a significantly lower dissolved oxygen concentration than arable ponds (Table 2.3). None of the other comparisons was statistically significant, although nitrate and total nitrogen concentrations were higher in arable than pastoral ponds (Table 2.3). Arable streams had higher concentrations of total phosphorus, total nitrogen and nitrate than pastoral streams; however, none of these differences between land uses was statistically significant (Table 2.3).

In 2013, concentrations of total phosphorus, biological oxygen demand and ammoniacal nitrogen were significantly higher in pastoral ponds than arable ponds (Table 2.4). However, arable ponds had higher concentrations of total nitrogen (marginally significant) and nitrate (significant) than pastoral ponds (Table 2.4). Arable streams had higher concentrations of total phosphorus, total nitrogen, ammoniacal nitrogen, nitrate and nitrite than pastoral streams, but differences between land uses were only statistically significant for nitrite (Table 2.4).

Table 2.3 Mean ± SE and two-sample *t*-test physiochemical characteristics of water for the study sites in **2011**, collected by the WFF Project. Natural logarithm transformations (ln(x)) were used where necessary. Significant differences are highlighted in bold ($p < 0.05$).

Habitat	**Land use**	**Temperature** (°C)	**pH**	**Conductivity** (µS/cm)	**Dissolved Oxygen** (mg/l)	**Total Phosphorus** (mg/l)	**Total Nitrogen** (mg/l)	**Nitrate as N** (mg/l)	**Sodium** (mg/l)	**Potassium** (mg/l)	**Calcium** (mg/l)	**Magnesium** (mg/l)
Ponds	**Arable**	11.77 ± 1.41	7.74 ± 0.13	588.5 ± 94.1	14.04 ± 2.13	0.080 ± 0.03	3.09 ± 0.88	5.67 ± 1.78	16.23 ± 5.24	3.52 ± 1.02	21.58 ± 1.24	5.82 ± 0.90
	Pastoral	10.05 ± 1.28	7.48 ± 0.26	695.2 ± 103.3	7.38 ± 1.22	0.279 ± 0.09	2.48 ± 0.41	2.83 ± 0.17	25.63 ± 7.49	11.82 ± 3.88	21.85 ± 1.38	8.70 ± 1.63
Two-sample *t*-test *df = 10*		t = 0.90 NSD	t = 0.90 NSD	t = 0.76 NSD	t = 2.71 **$p < 0.05$**	t = 2.48 **$p < 0.05$**	t = 0.29 NSD	t = 1.57 NSD	t = 1.18 NSD	t = 1.80 NSD	t = 0.14 NSD	t = 1.55 NSD
Streams	**Arable**	9.62 ± 0.55	8.22 ± 0.09	706.2 ± 52.1	11.31 ± 0.28	0.115 ± 0.06	7.77 ± 2.39	12.17 ± 3.70	14.72 ± 2.10	3.58 ± 0.99	26.95 ± 1.66	7.85 ± 0.75
	Pastoral	10.63 ± 0.91	8.19 ± 0.19	770.2 ± 76.9	9.91 ± 0.97	0.063 ± 0.02	5.59 ± 27.9	9.00 ± 3.62	20.78 ± 2.85	5.28 ± 2.61	27.25 ± 1.22	9.50 ± 0.98
Two-sample *t*-test *df = 10*		t = 0.96 NSD	t = 0.16 NSD	t = 0.69 NSD	t = 1.29 NSD	t = 0.71 NSD	t = 1.09 NSD	t = 0.88 NSD	t = 1.71 NSD	t = 0.09 NSD	t = 0.15 NSD	t = 1.34 NSD

NSD = no significant difference ($p > 0.05$).

Table 2.4 Mean ± SE and two-sample *t*-test physiochemical characteristics of water for the study sites in **2013**, collected by the WFF Project. Natural logarithm transformations (ln(x)) were used where necessary. Significant differences are highlighted in bold ($p < 0.05$).

Habitat	**Land use**	**pH**	**Conductivity** (µS/cm)	**Biological Oxygen Demand** (mg/l)	**Total Phosphorus** (mg/l)	**Total Nitrogen** (mg/l)	**Ammoniacal nitrogen as N** (mg/l)	**Nitrate as N** (mg/l)	**Nitrite as N** (mg/l)	**Sodium** (mg/l)	**Calcium** (mg/l)	**Magnesium** (mg/l)
Ponds	**Arable**	7.54 ± 0.20	456.3 ± 80.74	1.96 ± 0.43	0.063 ± 0.02	6.117 ± 1.89	0.060 ± 0.02	5.664 ± 2.07	0.012 ± 0.00	13.57 ± 4.60	93.08 ± 21.38	5.31 ± 0.62
	Pastoral	7.48 ± 0.16	398.7 ± 83.35	5.74 ± 0.90	0.339 ± 0.05	2.337 ± 0.38	0.312 ± 0.12	0.234 ± 0.03	0.012 ± 0.01	13.11 ± 4.68	65.78 ± 16.89	5.82 ± 1.46
Two-sample *t*-test *df = 10*		t = 0.23 NSD	t = 0.50 NSD	t = 3.78 ***p* < 0.01**	t = 5.41 ***p* < 0.001**	t = 2.08 ***p* = 0.06**	t = 2.38 ***p* < 0.05**	t = 6.70 ***p* < 0.001**	t = 0.72 NSD	t = 0.52 NSD	t = 1.00 NSD	t = 0.32 NSD
Streams	**Arable**	7.88 ± 0.15	584.0 ± 32.82	2.21 ± 0.39	0.129 ± 0.05	9.092 ± 1.76	0.110 ± 0.04	8.242 ± 1.81	0.013 ± 0.00	12.44 ± 2.78	118.0 ± 9.98	7.06 ± 1.04
	Pastoral	7.74 ± 0.20	692.6 ± 50.27	1.38 ± 0.38	0.035 ± 0.01	5.880 ± 2.48	0.030 ± 0.00	5.640 ± 2.45	0.004 ± 0.00	26.78 ± 5.31	134.8 ± 8.08	9.15 ± 0.96
Two-sample *t*-test * *df = 9*		t = 0.56 NSD	t = 1.87 NSD	t = 1.53 NSD	t = 1.76 NSD	t = 1.08 NSD	t = 1.91 NSD	t = 0.87 NSD	t = 2.72 ***p* < 0.05**	t = 2.52 ***p* < 0.05**	t = 1.27 NSD	t = 1.45 NSD

NSD = no significant difference ($p > 0.05$).

* The pastoral stream PS2 was omitted from the analysis due to lack of chemical data.

2.4. Conclusion

Freshwater habitats in many agricultural landscapes in the UK are affected by how adjacent land is managed. The two major land uses in agricultural catchments, arable and pastoral, differ in their management and practices. The twenty four pond and stream sites used in the current study represent the two different agricultural land uses, which are the main interest of this study. Categorising study sites based on the locations of sites within arable or pastoral land use area was straightforward; however, the influences of both land uses should be taken into account when assessing sites located near the boundary of two land use types.

The estimation of site-specific land use and water flow direction for study sites presented in this chapter investigated the possible impact from surrounding agricultural fields. The final classification of sites was developed based on surrounding land use and source of water flow. Twenty two sites were classified as reflecting their land use impact. Two sites (one pond and one stream), classified as ‘mixed’, are likely to receive runoff and contamination from both land use types. However, because both were located in arable land use they were retained in the overall analysis but their characteristics (physicochemical and biological) were checked against other arable sites and no significant differences were found. The analysis used here is a good model in determining the impact of agriculture for many other arable-pastoral land use catchments.

Pastoral ponds were mainly associated with high concentrations of total phosphorus and ammonia and low concentrations of dissolved oxygen whereas arable ponds had high concentrations of total nitrogen and nitrate. Arable streams had generally higher concentrations of total nitrogen, total phosphorous, nitrate and nitrite than pastoral streams.

Chapter 3: The effects of different types of agricultural land use on freshwater macroinvertebrate communities

3.1. Introduction

Agricultural landscapes are managed for crop and animal production. However, agriculture may be also a major contributor of pollution to freshwater ecosystems (Allan, 2004; Kyriakeas & Watzin, 2006), resulting in a significant decrease in water quality and habitat degradation (Allan, 2004; Dudgeon *et al.*, 2006). For example, a study conducted on Irish rivers and streams found that intensive crop production and cattle grazing, along with urbanisation, were the main factors affecting water quality and ecological status (Donohue *et al.*, 2006). Increased agricultural land use causes many changes in freshwater habitats, including elevated nutrient concentrations and accumulation of fine sediments, as well as increased temperature and light as a result of riparian vegetation removal (Haygarth & Jarvis, 2002; Allan, 2004). The impacts of agriculture on the physical and chemical characteristics of freshwater ecosystems result in habitat degradation and alterations in resource availability to aquatic organisms and therefore affect the structure and functions of biological communities.

Benthic macroinvertebrates are important components of aquatic ecosystems (Grant, 2002), providing food sources for many aquatic and terrestrial vertebrates such as birds, fishes, amphibians and reptiles (Covich *et al.*, 1999; Suter & Cormier, 2015). They are often classified, based on feeding mechanisms, into functional feeding groups: shredders (feed on living or decomposing vascular plant tissue), collectors (feed on fine detrital particles), scrapers (feed on attached algae) and predators (feed on live prey) (Cummins, 1973). Aquatic invertebrates play an important role in the functioning of freshwater ecosystems (Wallace & Webster, 1996; Covich *et al.*, 2004) and underpin many ecosystem services (Suter & Cormier, 2015). For example, shredder invertebrates play a key role in decomposition processes and hence affect nutrient cycling (Wallace & Webster, 1996; Graça, 2001; Grant, 2002; Rasmussen *et al.*, 2012); grazing invertebrates influence the structure and abundance of algal communities (Wallace & Webster, 1996; Covich *et al.*, 1999; Graça, 2001), which are important for nutrient removal and water purification (Cardinale, 2011); and some invertebrates (e.g. odonates and mayflies) have aesthetic value (Suter & Cormier, 2015). In addition, aquatic macroinvertebrates have been used as a good indicator for assessing water quality (Rosenberg & Resh, 1993) because they are diverse communities, easily sampled compared to fish and have many sensitive taxa (Suter & Cormier, 2015).

Several studies have investigated the effects of agriculture on aquatic macroinvertebrate communities in ponds (Trigal *et al.*, 2007; Céréghino *et al.*, 2008; Ruggiero *et al.*, 2008; Della Bella & Mancini, 2009; Czerniawski *et al.*, 2013; Fuentes-Rodriguez *et al.*, 2013) and streams (Dolédec *et al.*, 2006; Kyriakeas & Watzin, 2006; Piggott *et al.*, 2012; Riens *et al.*, 2013; Lange *et al.*, 2014). For example, Trigal *et al.* (2007) showed that the best predictor of macroinvertebrate community structure in Mediterranean ponds was human disturbance (mainly agricultural pressure) whereas other biotic and habitat factors were of secondary importance. Dolédec *et al.* (2006) found that changes in the relative abundances of stream invertebrate communities were strongly associated with land use (livestock grazing), and several studies have reported a significant decrease in the total number of macroinvertebrates in sites affected by agriculture (Trigal *et al.*, 2007; Della Bella & Mancini, 2009; Riens *et al.*, 2013). In addition, high concentrations of phosphorus in agricultural ponds were associated with reduced diversity of macroinvertebrate communities (Fuentes-Rodriguez *et al.*, 2013).

It is widely recognised that streams and ponds have different physical and chemical characteristics and hence differ in their biodiversity. Ponds are characterized by small catchment areas and therefore each pond has its individual physiochemical conditions that vary markedly between ponds (Williams *et al.*, 2004). In contrast, streams cover large catchment areas and have connecting drainage, and thus they are less variable in their physical and chemical characteristics (Williams *et al.*, 2004). A comparison of biodiversity between freshwater habitats in an agricultural landscape in Southern England revealed that local species richness (alpha diversity) for macrophytes and macroinvertebrates was the highest in rivers followed by ponds, streams and ditches whereas regional species richness (gamma diversity) was highest for ponds (Williams *et al.*, 2004). In addition, a similar comparison found that streams and rivers had highest proportions of taxa sensitive to pesticides (mayflies, stoneflies and amphipod crustaceans) than pond or ditch taxa (Biggs *et al.*, 2007). However, little is known about how different water bodies respond to changes caused by different types of agricultural land use.

Most research on the impacts of agriculture on freshwater ecosystems has focused on agricultural activities in general, whether crop production or grassland grazing, and usually compared agricultural to reference sites (Trigal *et al.*, 2007; Céréghino *et al.*, 2008; Ruggiero *et al.*, 2008; Gücker *et al.*, 2009; Fuentes-Rodriguez *et al.*, 2013; Riens *et al.*, 2013). Some more focused studies have investigated the effects of improving grassland for livestock grazing (Dolédec *et al.*, 2006; Lange *et al.*, 2014) on freshwater ecosystems but little research has focused on the effects of different types of agricultural practices on aquatic communities. Kyriakeas and Watzin (2006) compared stream benthic macroinvertebrate communities in reference sites and two types of agricultural practices in the USA: corn fields and dairy cattle pasture. They concluded that both agricultural practices were more impacted than reference sites, and the sites where cattle had access was more impacted than corn fields. However, little is known about the impacts of different agricultural land uses (e.g. arable farming and livestock grazing) on macroinvertebrate communities in different water habitats (e.g. ponds and streams).

The aim of this chapter is to investigate the impact of two agricultural land uses (arable and pastoral) on freshwater macroinvertebrate communities in ponds and streams. The objectives of this study were to compare the structure (total abundance, taxonomic richness and diversity), functional role (functional feeding groups) and community composition of macroinvertebrates in ponds and streams located in arable and pastoral agricultural land use.

3.2. Methods

3.2.1. Study sites

Study sites were ponds and streams located in agricultural landscapes in Eye Brook and Stonton Brook catchments in Leicestershire. They include 6 arable and 6 pastoral ponds as well as 6 arable and 6 pastoral streams (described in detail in Chapter 2). Physiochemical characteristics of pond and stream water were determined in April 2011 by members of the Water Friendly Farming (WFF) project (Biggs *et al.*, 2014).

3.2.2. Macroinvertebrate sorting and identification

Benthic macroinvertebrate samples were collected in spring 2011 by the WFF project (Biggs *et al.*, 2014), but processed and identified as part of the current project. Each site was sampled for 3 minutes using a 1-mm mesh hand-net (frame 0.26 m × 0.30 m), the sampling time being divided equally between major habitat types (Williams *et al.*, 2004). All samples were preserved in 70% industrial methylated spirits (IMS) until the sorting could be performed.

Sample processing involved using tap water to wash each sample through a nest of sieves (1 mm and 500 µm) to remove IMS and sediments. The contents of each sieve were then placed in white sorting trays and invertebrates were picked out, sorted and placed in 70% IMS. All invertebrates were examined under a microscope at 40 × magnification and identified to family levels using appropriate keys (Macan, 1959; Merritt *et al.*, 2008; Pawley *et al.*, 2011; Dobson *et al.*, 2012) and counted. Taxa were assigned to functional feeding groups (i.e. collectors, predators, scrapers, shredders) following Merritt *et al.* (2008).

3.2.3. Data analysis

Principal component analysis (PCA) on the covariance matrix was used to investigate how the physicochemical characteristics of pond and stream water varied with land use. Chemical parameters were natural logarithm transformed before analysis.

Macroinvertebrate communities were analysed using the following metrics: total abundance of macroinvertebrates, taxonomic richness, Shannon diversity index (using $\log_{10}$), taxonomic evenness, relative abundance of major taxonomic groups, abundances of functional feeding groups. Metrics were compared between arable and pastoral sites using two-sample *t*-tests and analyses were performed separately for ponds and streams. The total abundance of macroinvertebrates and the abundances of functional feeding groups were natural logarithm transformed before analyses where necessary. The relative abundances of major taxonomic groups were arcsine square root transformed before analysis.

The response of macroinvertebrate communities in ponds and streams to land use was investigated using PCA. Taxa were removed from the analysis if they constituted less than 1% of the overall abundance. Analysis of Similarity (ANOSIM) (Clarke, 1993) was used to examine whether there were significant differences in the macroinvertebrate community compositions between arable and pastoral land uses in ponds and streams. Similarity Percentage Analysis (SIMPER) (Clarke, 1993) was used to determine which taxonomic groups were responsible for differences between communities in different land uses and calculate the percentage contribution of each taxon to the overall dissimilarity (Bray-Curtis). Abundances of macroinvertebrate taxa were ln (x+1) transformed before analyses. Statistical analyses were carried out using R (R Core Team, 2013). In R, the package ggplot2 (Wickham, 2009) was used for producing graphics, and the package vegan (Oksanen *et al.*, 2013) was used for running ANOISM and SIMPER analyses.

3.3. Results

3.3.1. Physicochemical characteristics of freshwater habitats

The results of principal component analysis of the physicochemical characteristics of water in ponds and streams are shown in Figure 3.1. For ponds, the first (PC1) and second (PC2) components explained 55.6% of the total variation, and arable and pastoral ponds were separated along PC1 (Figure 3.1a). Pastoral ponds were associated with higher concentrations of total phosphorus (TP), potassium, magnesium and sodium, whereas arable ponds were associated with increased pH, dissolved oxygen (DO), total nitrogen (TN) and nitrate. In streams, PC1 and PC2 explained 63.7% of the total variation and whereas arable streams were tightly clustered along PC1, pastoral streams were much more variable (Figure 3.1b). Arable streams had higher concentrations of TP, pH and DO. Furthermore, arable streams and two pastoral streams (PS3 and PS6), were characterized by higher concentrations of TN and nitrate. The pond (AP5) and the stream (AS5) which were classified as ‘mixed’ sites (Chapter 2) did not differ from the rest of other arable sites in terms of physicochemical characteristics of water.

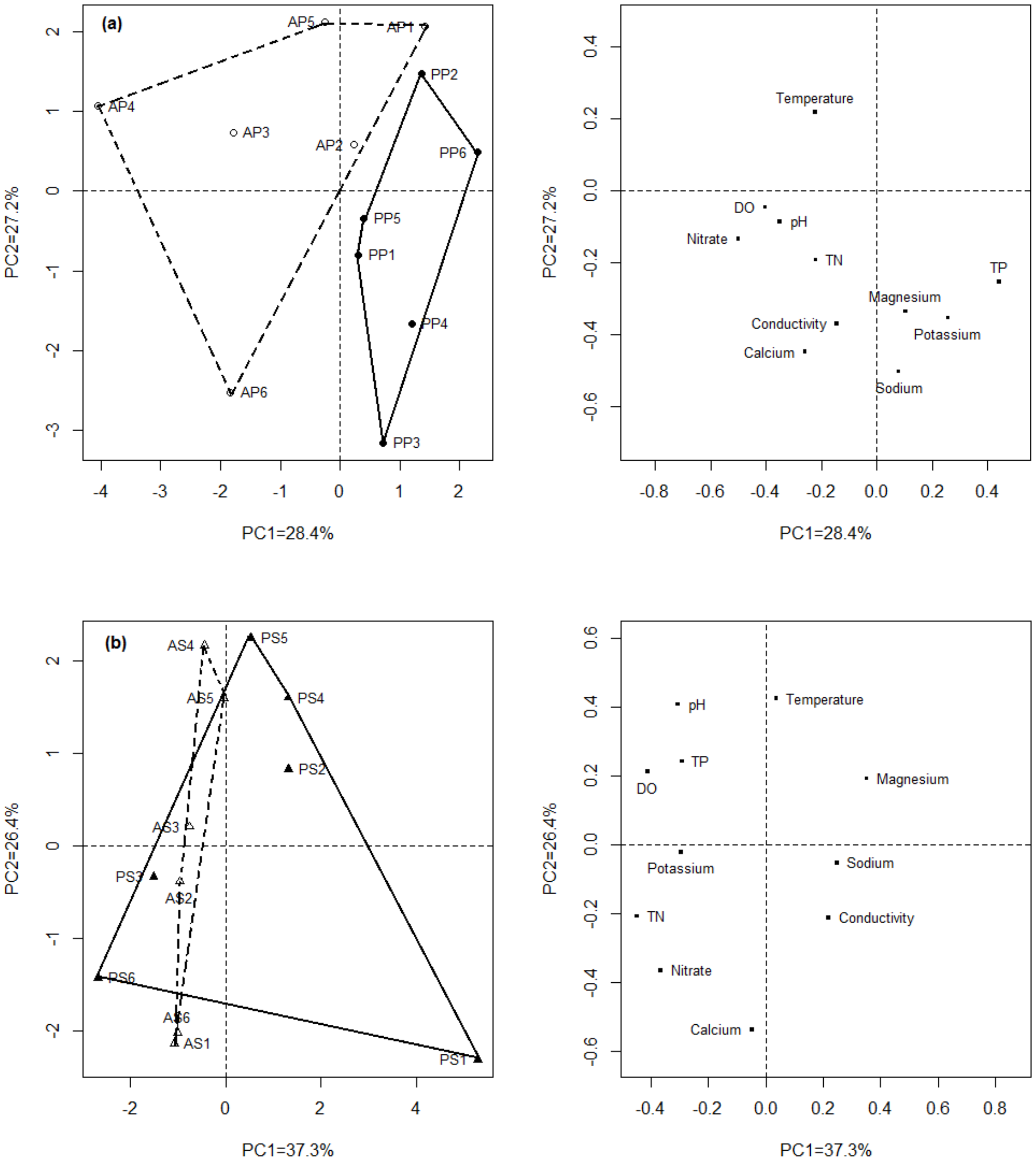

Figure 3.1 Principal component analysis of the physicochemical characteristics of water in (a) ponds and (b) streams in arable (open circles and triangles and dashed lines) and pastoral (closed circles and triangles and solid lines) sites.

3.3.2. Total abundance, taxonomic richness and diversity of macroinvertebrates

A total of 55652 macroinvertebrates from 66 taxa were identified from the 24 study sites. Forty taxa were found in both pond and stream samples, nineteen taxa were found in ponds only and seven taxa were found in streams only (Table 3.1). The total number of invertebrates in ponds ranged from 480 to 6064 individuals for arable sites, and from 445 to 4644 individuals for pastoral sites. In streams, the total number of invertebrates ranged from 861 to 2343 and from 1326 to 9342 individuals for arable and pastoral sites, respectively. There was no significant effect of land use on the total abundance of macroinvertebrates in ponds (two-sample *t*-test: $t_{10} = 0.85$, $p > 0.05$; Figure 3.2a) but pastoral streams had significantly more invertebrates than arable streams (two-sample *t*-test: $t_{10} = 2.34$, $p < 0.05$; Figure 3.2b). Similarly, whereas there was no significant effect of land use on the richness of macroinvertebrates in ponds (two-sample *t*-test: $t_{10} = 1.02$, $p > 0.05$; Figure 3.2c), arable streams had fewer taxa than in pastoral streams (two-sample *t*-test: $t_{10} = 2.94$, $p < 0.05$; Figure 3.2d).

Macroinvertebrate diversity, measured as Shannon index, was higher in pastoral ponds than arable ponds (Figure 3.2e), but this difference was not statistically significant (two-sample *t*-test: $t_{10} = 0.41$, $p > 0.05$). In streams, diversity was also similar between arable and pastoral sites (two-sample *t*-test: $t_{10} = 0.07$, $p > 0.05$; Figure 3.2f). There was no significant effect of land use on the taxonomic evenness of macroinvertebrate communities in either ponds or streams (two-sample *t*-test; ponds: $t_{10} = 0.91$, $p > 0.05$; streams: $t_{10} = 0.33$, $p > 0.05$).

Table 3.1 Macroinvertebrate taxa identified in ponds and streams.

No.	Ponds and streams		Ponds only		Streams only	
	Taxa	Family	Taxa	Family	Taxa	Family
1	Tricladida	Planariidae *Polycelis*	Gastropoda	Bithyniidae	Ephemeroptera	Ephemeridae
2	Gastropoda	Hydrobiidae	Gastropoda	Planorbidae	Hemiptera	Nepidae
3	Gastropoda	Lymnaeidae	Amphipoda	Crangonyctidae	Trichoptera	Goeridae
4	Bivalvia	Sphaeriidae	Ephemeroptera	Caenidae	Trichoptera	Lepidostomatidae
5	Rhynchobdella	Glossiphoniidae	Odonata	Libellulidae	Megaloptera	Sialidae
6	Pharyngobdella	Erpobdellidae	Hemiptera	Corixidae	Diptera	Pedicidae
7	Oligochaeta		Hemiptera	Gerridae	Diptera	Simuliidae
8	Arachnida	Hydrachnida	Hemiptera	Hydrometridae		
9	Isopoda	Asellidae	Hemiptera	Naucoridae		
10	Amphipoda	Gammaridae	Hemiptera	Notonectidae		
11	Ephemeroptera	Baetidae	Hemiptera	Pleidae		
12	Ephemeroptera	Leptophlebiidae	Coleoptera	Haliplidae		
13	Plecoptera	Nemouridae	Coleoptera	Noteridae		
14	Odonata	Coenagrionidae	Trichoptera	Hydroptilidae		
15	Odonata	Aeshnidae	Trichoptera	Hydropsychidae		
16	Hemiptera	Veliidae	Trichoptera	Molannidae		
17	Coleoptera	Curculionidae	Diptera	Culicidae		
18	Coleoptera	Dytiscidae	Diptera	Syrphidae		
19	Coleoptera	Elmidae	Diptera	Tabanidae		
20	Coleoptera	Helophoridae				
21	Coleoptera	Hydraenidae				
22	Coleoptera	Hydrophilidae				
23	Coleoptera	Scirtidae Larvae				
24	Trichoptera	Beraeidae				
25	Trichoptera	Glossosomatidae				
26	Trichoptera	Leptoceridae				
27	Trichoptera	Limnephilidae				
28	Trichoptera	Polycentropodidae				
29	Trichoptera	Psychomyiidae				
30	Diptera	Ceratopogonidae				
31	Diptera	Chaoboridae				
32	Diptera	Chironomidae				
33	Diptera	Dixidae				
34	Diptera	Empididae				
35	Diptera	Limoniidae				
36	Diptera	Psychodidae				
37	Diptera	Ptychopteridae				
38	Diptera	Stratiomyidae				
39	Diptera	Tipulidae				
40	Lepidoptera					

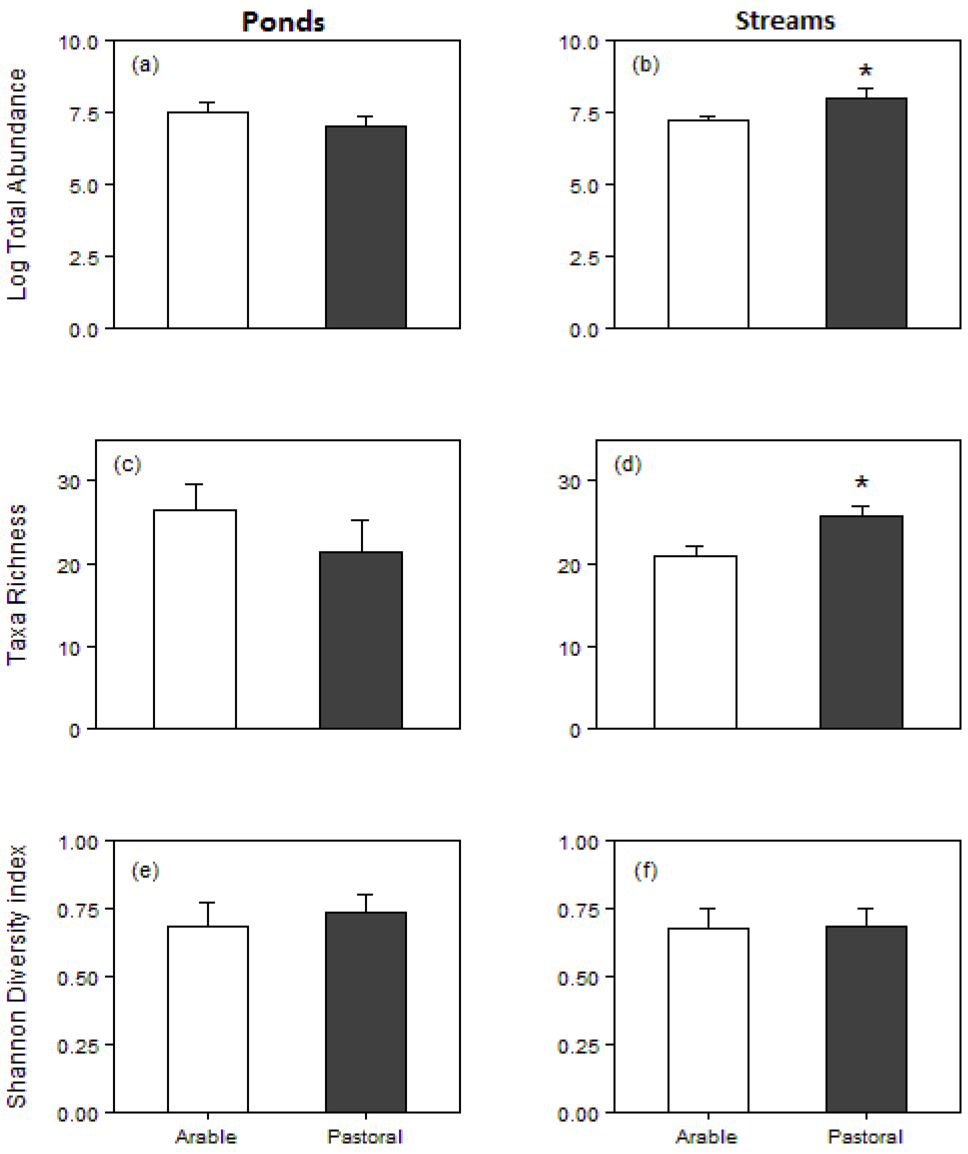

Figure 3.2 Mean (+ SE) macroinvertebrate total abundance (total number of individuals) (a, b), taxonomic richness (c, d) and Shannon diversity index (e, f) in arable (white bars) and pastoral (black bars) in ponds on the left side and streams on the right side. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

3.3.3. Relative abundance of major macroinvertebrate groups

There was a marked difference in the relative abundance of different taxonomic groups between arable and pastoral ponds. Diptera were the dominant group in both types of ponds, but whereas they accounted for 62% of individuals in arable ponds (Figure 3.3a), they accounted for only 25% of individuals in pastoral ponds (Figure 3.3b). In contrast, Mollusca and Coleoptera were more abundant in pastoral than arable ponds (Figure 3.3b).

Arable and pastoral streams also differed in the relative abundance of different taxonomic groups (Figure 3.3c and 3.3d). Amphipoda were the dominant group in both arable and pastoral streams, accounting for 32% and 42% of individuals, respectively. Similarly, Diptera were abundant in both types of streams and they accounted for 23% of individuals in arable streams and 19% of individuals in pastoral streams. However, Mollusca were more abundant in pastoral streams and accounted for 24% of individuals.

There was almost a twofold difference in the relative abundance of Diptera in arable and pastoral ponds, and this difference was statistically significant (two-sample *t*-test: $t_{10} = 2.66$, $p < 0.05$; Figure 3.4). The relative abundances of Coleoptera, Mollusca and Oligochaeta were higher in pastoral ponds than arable ponds, but these differences were only marginally significant for Coleoptera (two-sample *t*-test: $t_{10} = 2.01$, $p = 0.07$; Figure 3.4).

Amphipoda, Mollusca and Plecoptera were more abundant in pastoral streams than in arable streams, but these differences between land uses were only significant for Plecoptera (two-sample *t*-test: $t_{10} = 2.24$, $p < 0.05$; Figure 3.5). The relative abundances of Diptera, Ephemeroptera and Oligochaeta were higher in arable streams than pastoral streams, but these differences between land uses were only statistically significant for Oligochaeta (two-sample *t*-test: $t_{10} = 2.26$, $p < 0.05$, Figure 3.5). Actual abundances for individual taxa sampled at each site are presented in Appendix A, Table A1 for pond taxa and Table A2 for stream taxa.

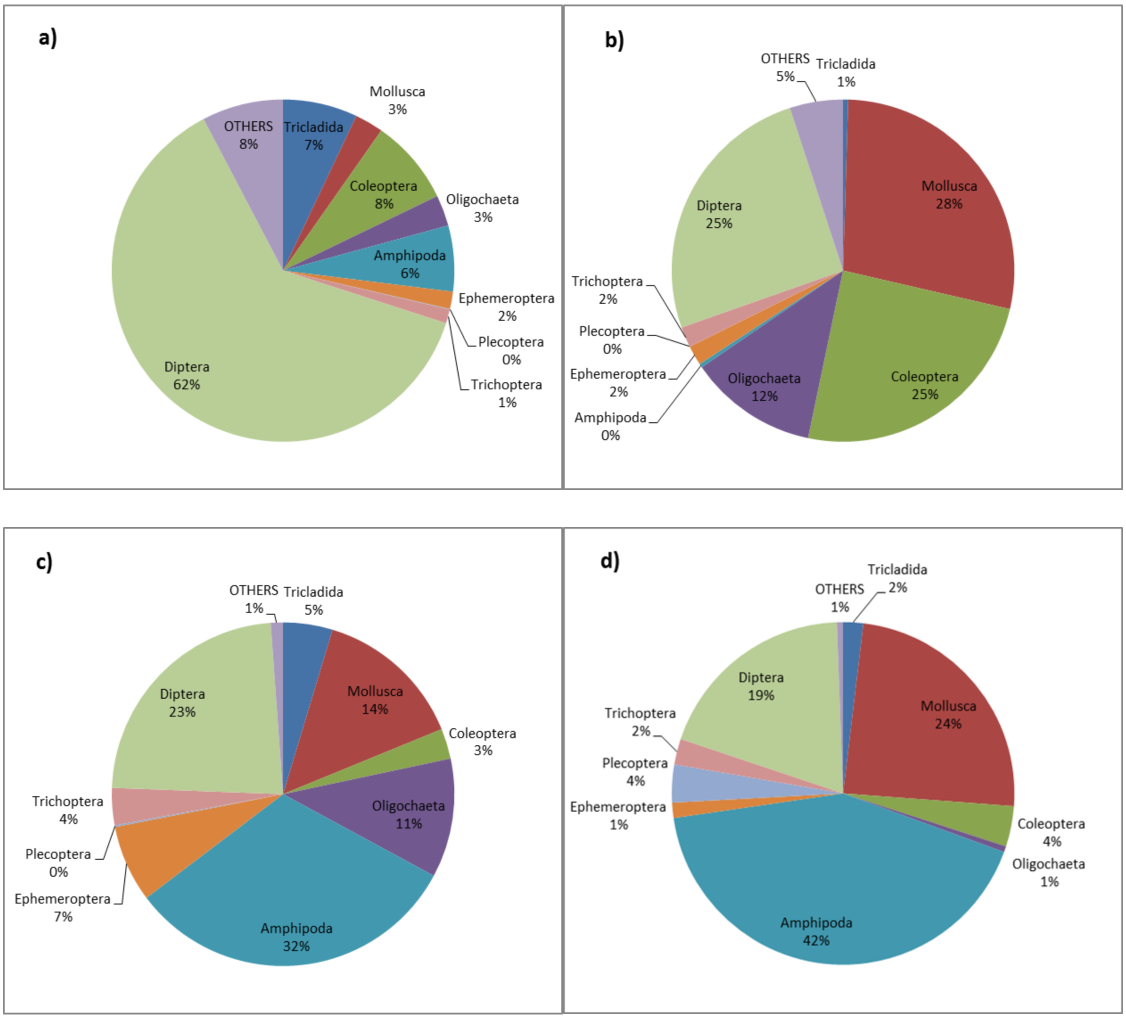

Figure 3.3 Relative abundance (%) of major macroinvertebrate groups found in (a) arable ponds, (b) pastoral ponds, (c) arable streams and (d) pastoral streams.

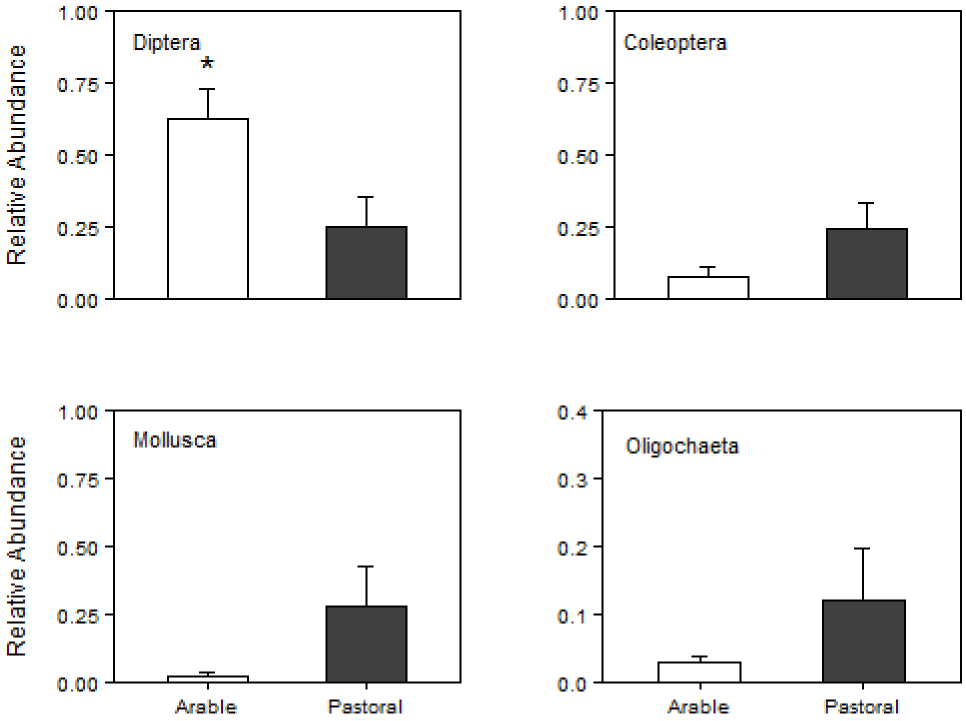

Figure 3.4 Mean (+ SE) relative abundance of major macroinvertebrate groups in **ponds** in arable (white bars) and pastoral (black bars) sites. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

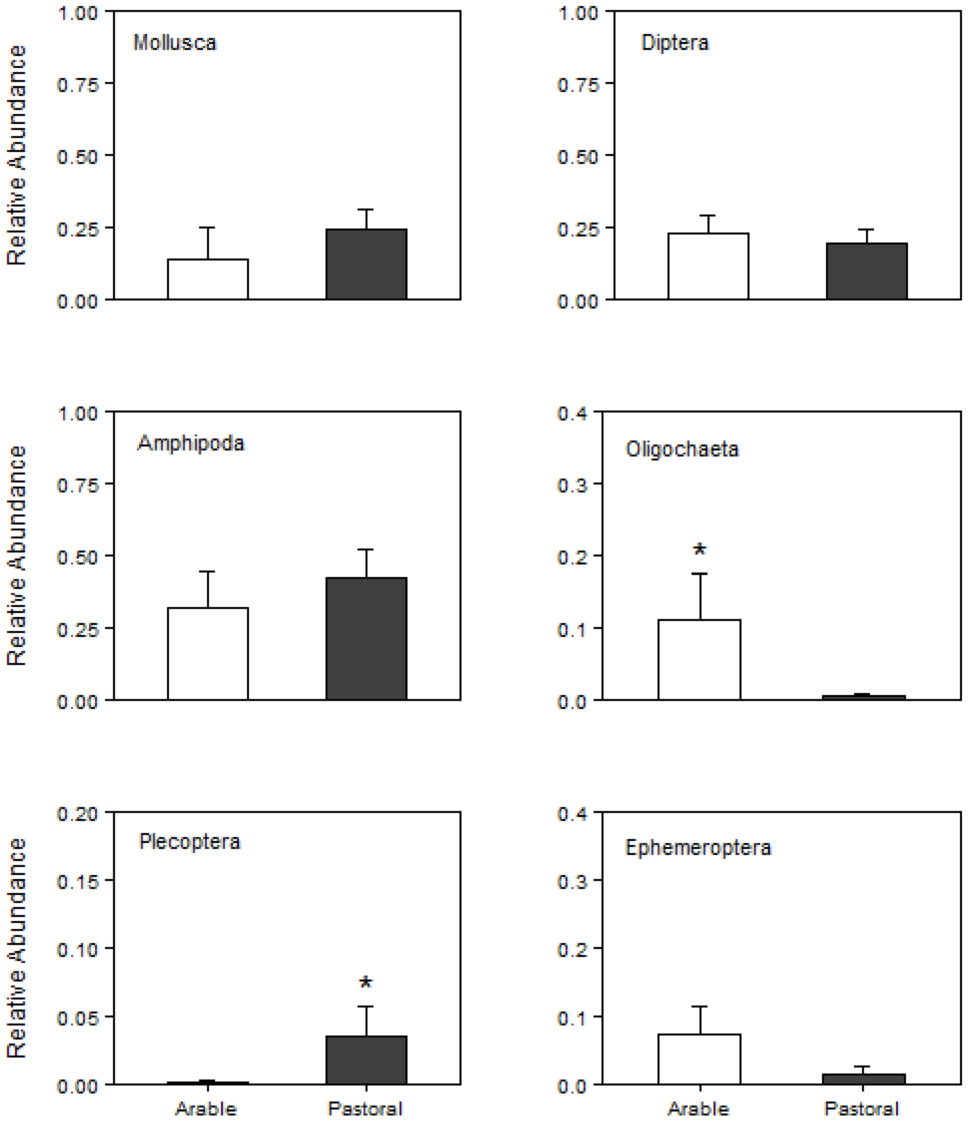

Figure 3.5 Mean (+ SE) relative abundance of major macroinvertebrate groups in **streams** in arable (white bars) and pastoral (black bars) sites. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

A comparison of the abundances of functional feeding groups showed that scrapers and shredders were more abundant in pastoral water bodies than in arable water bodies (Figure 3.6a and 3.6b). However, whereas these differences were marginally significant for scrapers (two-sample *t*-test: $t_{10} = 1.91$, $p = 0.09$) and significant for shredders (two-sample *t*-test: $t_{10} = 2.55$, $p < 0.05$) in streams, there were no significant differences in the abundance of shredders or scrapers in ponds. Moreover, there was no significant difference in collector or predator abundances between the two land uses for either ponds or streams.

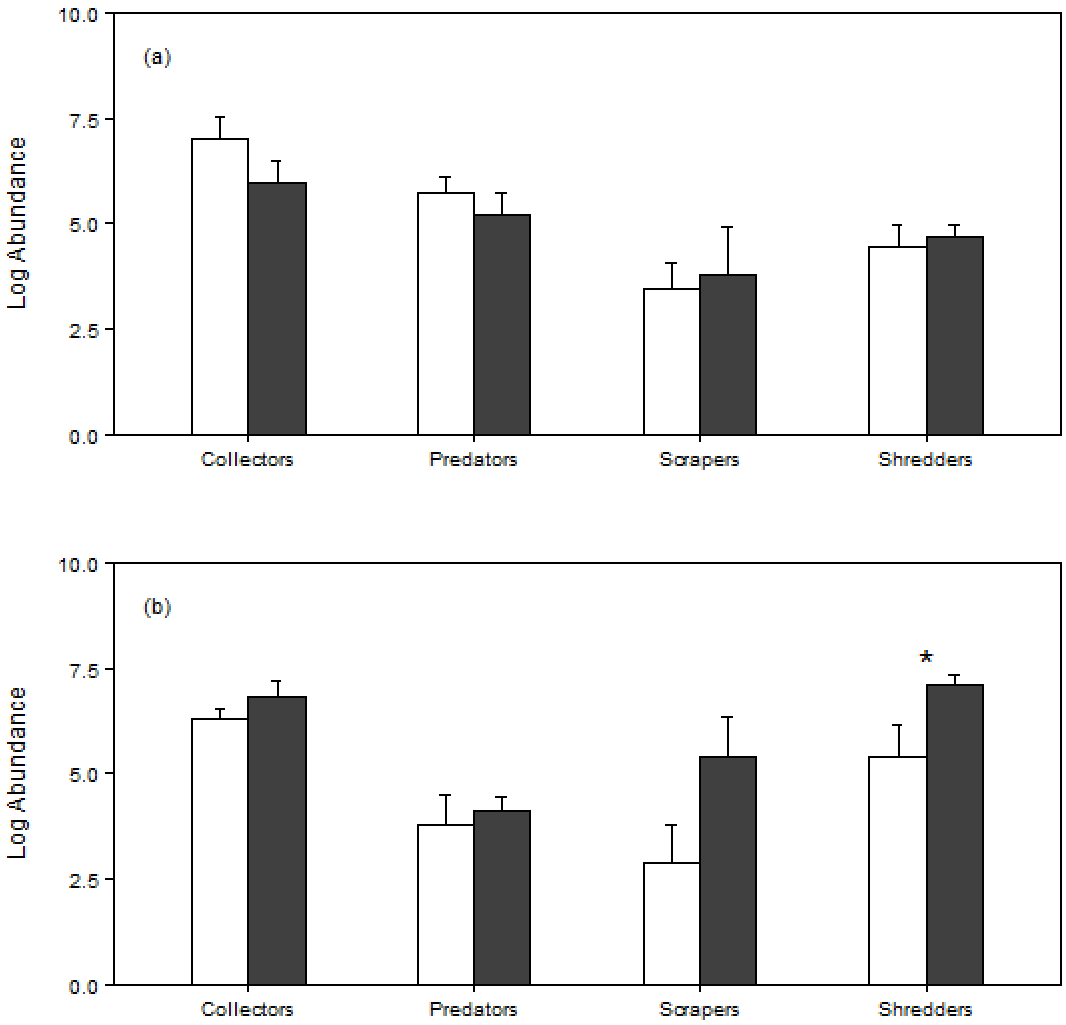

Figure 3.6 Mean (+ SE) abundance (total number of individuals) of functional feeding groups in arable (white bars) and pastoral (black bars) in (a) ponds and (b) streams. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

3.3.4. Macroinvertebrate community composition

The results of the PCA for macroinvertebrate communities in ponds and streams are presented in Figure 3.7. In ponds, the first two components explained 58.8% of the total variation and there was a strong overlap between arable and pastoral ponds (Figure 3.7a). There was greater variation in arable ponds along PC1 than PC2, whereas the opposite was true for pastoral ponds. Arable ponds were characterised by high (AP3, AP4, AP6) or low (AP1, AP2, AP5) abundances of Lymnaeidae, Planorbidae, Gammaridae, Coenagrionidae, Corixidae, Chironomidae and Baetidae whereas pastoral ponds were characterised by high (PP1, PP3) or low (PP2, PP4, PP5) abundances of with Hydrophilidae, Dytiscidae and Planariidae. For the stream macroinvertebrates, PC1 and PC2 explained 58.8% of the total variation, and arable and pastoral streams separated along PC1 (Figure 3.7b). The most abundant taxa in arable streams were Oligochaete, Ceratopogonidae, Gammaridae, Sphaeriidae and Baetidae, whereas pastoral streams were associated with higher abundances of Hydrobiidae, Nemouridae, Gammaridae, Limnephilidae, Scirtidae and Simuliidae. The ‘mixed’ sites (AP5) and (AS5) (Chapter 2) did not differ from the rest of other arable sites in terms of invertebrate community composition.

The results of analysis of similarity (ANOSIM) revealed that the macroinvertebrate communities in ponds were not significantly different between arable and pastoral sites ($R = 0.07$, $p > 0.5$), but there was a significant difference in macroinvertebrate communities between arable and pastoral streams ($R = 0.2$, $p = 0.02$). The results of similarity percentages (SIMPER) analysis are presented in Table 3.2. In ponds, the taxonomic groups Planorbidae, Planariidae, Chironomidae, Corixidae, Coenagrionidae, Lymnaeidae and Baetidae ordered as the top taxa and accounted for 51% of dissimilarities in distinguishing between the two land uses. All these taxa were more abundant in arable ponds except Planorbidae. SIMPER analysis for stream macroinvertebrates detected the top five taxa which contributed to 54.5% of dissimilarities between arable and pastoral land uses. These taxa were Hydrobiidae, Nemouridae, Planariidae, which were higher in pastoral streams plus Oligochaeta and Baetidae, which were more abundant in arable streams.

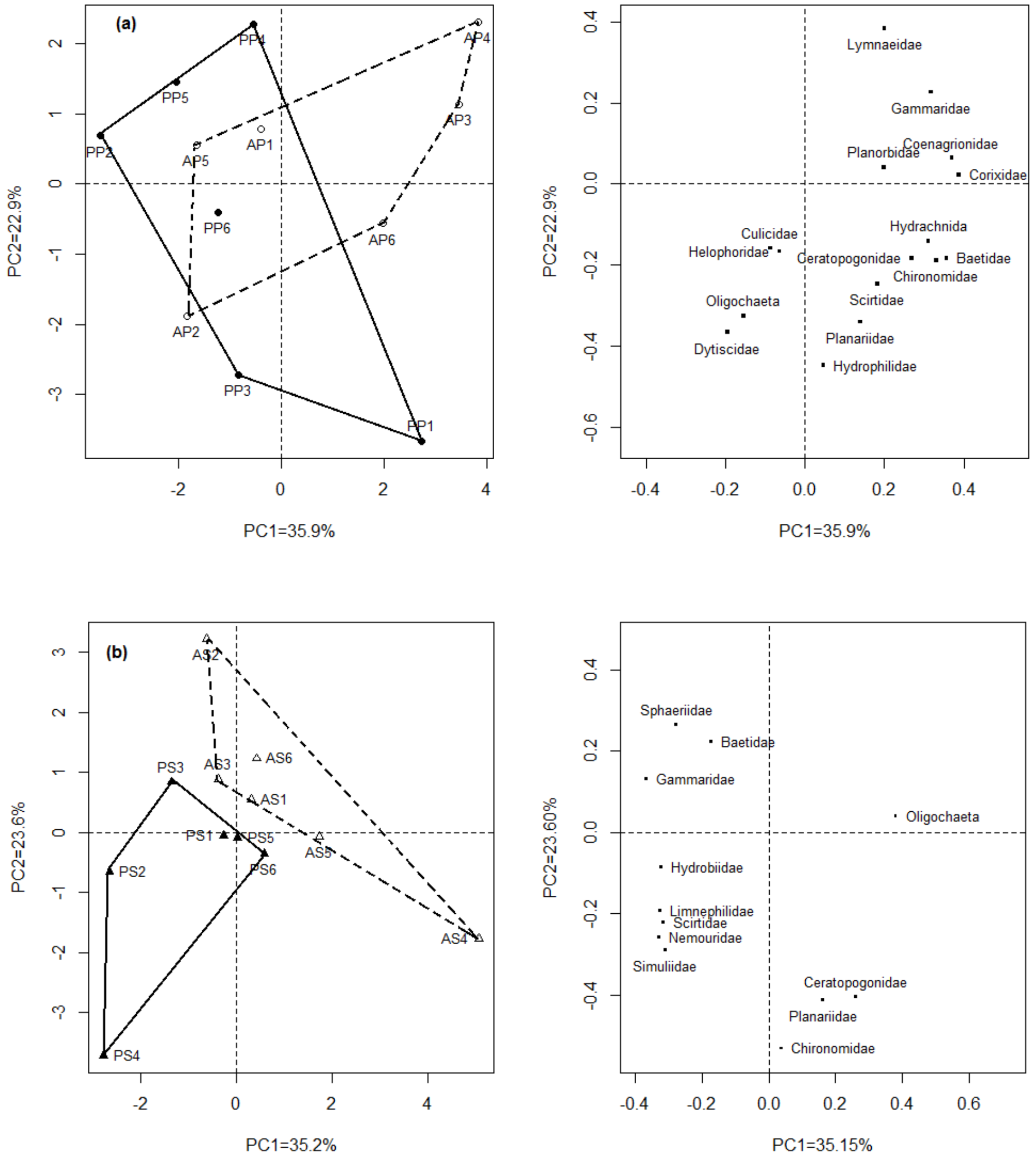

Figure 3.7 Principal component analysis of the macroinvertebrate families in (a) ponds and (b) streams in arable (open circles and triangles and dashed lines) and pastoral (closed circles and triangles and solid lines) sites.

Table 3.2 Similarity of percentages (SIMPER) analysis of macroinvertebrate communities using Bray-Curtis dissimilarity between arable and pastoral ponds or streams. Contribution % is percent contribution a taxon makes to the overall dissimilarity between the two communities. Cumulative % is the ordered percentage of cumulative contribution.

Taxa	**Contribution%**	**Cumulative%**	**Average Log Abundance**	
			Arable	Pastoral
Ponds				
Planorbidae	8.77	8.77	2.12	3.33
Planariidae	7.72	16.49	2.71	1.10
Chironomidae	7.59	24.08	6.73	4.86
Corixidae	7.45	31.53	2.93	1.21
Coenagrionidae	6.60	38.13	2.39	0.58
Lymnaeidae	6.42	44.55	2.61	1.13
Baetidae	6.42	50.97	2.11	1.80
Gammaridae	6.17	57.14	2.31	0.54
Ceratopogonidae	6.13	63.27	2.79	2.26
Helophoridae	6.06	69.33	1.98	3.91
Dytiscidae	5.82	75.14	3.16	4.23
Culicidae	5.79	80.93	2.43	1.31
Hydrachnida	5.73	86.66	2.11	1.68
Scirtidae	5.30	91.95	1.41	1.66
Oligochaeta	4.41	96.36	3.34	3.92
Hydrophilidae	3.64	100.00	2.48	3.06
Streams				
Hydrobiidae	14.34	14.34	1.97	5.08
Nemouridae	11.21	25.55	0.55	3.52
Planariidae	10.09	35.64	1.68	3.20
Oligochaeta	9.96	45.60	3.97	1.51
Baetidae	8.94	54.54	2.76	1.84
Scirtidae	8.08	62.62	2.25	4.23
Gammaridae	7.84	70.46	5.02	6.85
Sphaeriidae	7.43	77.89	3.33	4.00
Simuliidae	6.99	84.88	0.88	2.06
Ceratopogonidae	6.27	91.15	2.78	2.30
Limnephilidae	5.20	96.35	3.16	3.45
Chironomidae	3.65	100.00	5.20	5.79

3.4. Discussion

The objectives of this study were to compare the structure (total abundance, taxonomic richness and diversity), functional role (functional feeding groups) and community composition of macroinvertebrates in ponds and streams located in arable and pastoral agricultural land use. The total number of macroinvertebrates, taxonomic richness and diversity index did not significantly differ between arable and pastoral ponds. In contrast, although the total abundance and taxonomic richness of macroinvertebrates in streams were significantly higher in pastoral than arable sites, no significant difference in invertebrate diversity was found between the two land uses. There were no significant differences in the abundances of functional feeding groups between arable and pastoral ponds, but in streams scrapers and shredders were more abundant in pastoral sites than arable sites. Although the community composition of macroinvertebrates in ponds did not differ between arable and pastoral sites, arable ponds were associated with high abundance of Diptera (mainly Chrionomidae) and pastoral ponds had high abundances of Mollusca (mainly Lymnaeidae and Planorbidae) and Oligochaeta. Macroinvertebrate community compositions were significantly different between arable and pastoral streams; arable streams were associated with a significant increase in Oligochaete abundance and an almost total absence of Plecoptera (Nemouridae).

Agricultural land use can have a severe effect on the structure of aquatic macroinvertebrate communities (Dolédec *et al.*, 2006; Kyriakeas & Watzin, 2006; Trigal *et al.*, 2007; Della Bella & Mancini, 2009; Fuentes-Rodriguez *et al.*, 2013; Riens *et al.*, 2013). To the best of my knowledge, none of the studies that investigated the impact of agriculture on pond invertebrates has compared different agricultural practices; however, the general effect of agricultural land use on pond macroinvertebrates could be similar to that reported in other studies. In Mediterranean ponds located in agricultural landscapes, Della Bella and Mancini (2009) found that the total abundance of macroinvertebrates in reference ponds was significantly greater than both intermediate and degraded ponds.

In a study comparing stream macroinvertebrate communities in reference sites and two types of agricultural practices (corn fields and dairy cattle pasture), Kyriakeas and Watzin (2006) found that both types of agricultural sites were impacted compared to reference sites and sites where cattle had access were more affected than sites in corn fields. This does not appear to be the case in the current study where invertebrate communities seem to be more impacted in arable streams. However, cattle had unrestricted access to streams in the study by Kyriakeas and Watzin (2006), whereas most pastoral streams in this study are bordered by either fences or riparian buffers and hence no direct access for livestock. Similarly, Riens *et al.* (2013) found that buffered wetlands, located in an area dominated by agriculture, had significantly higher total numbers of benthic invertebrates and more diverse communities than non-buffered wetlands.

The relative abundance of scrapers and shredders are predicted to decrease with increasing human impact (Kerans & Karr, 1994). Comparing functional feeding groups in this study showed that the high abundance of molluscs (mainly gastropods) was reflected in the high abundance of scrapers in pastoral water bodies; and the high abundance of stoneflies and amphipods in pastoral streams led to high abundance of shredders. The findings of the current study are inconsistent with Kyriakeas and Watzin (2006) who found that streams located on pasture sites had significantly higher relative abundance of scrapers and lower relative abundance of shredders than cornfield sites. However, (Maloney & Feminella, 2006) reported that the use of functional feeding group metrics was not a good indicator of disturbance and commonly had high seasonal and annual variations, but the abundance of Chironomidae was a good indicator of disturbance and had little seasonal and annual differences. A further difficulty regarding the use of functional feeding groups is in accurately assigning invertebrate taxa to specific feeding groups (Cummins & Klug, 1979; Trigal *et al.*, 2007) and the possibility that some invertebrates may change their feeding behaviour during life (Trigal *et al.*, 2007).

Agricultural activities may cause changes in the community composition of benthic invertebrates. A general characteristic of impacted sites is an increase in tolerant taxa (e.g.

Diptera, particularly Chironomidae) and a decrease in sensitive taxa (e.g. stoneflies, mayflies and caddies flies) (Kerans & Karr, 1994; Kyriakeas & Watzin, 2006; Biggs *et al.*, 2007). In Mediterranean ponds surrounded by farmlands, Trigal *et al.* (2007) found that Chironomidae was the dominant taxa and their relative abundance reached up to 79% of the total individuals in ponds. In addition, Fuentes-Rodriguez *et al.* (2013) reported that Chironomidae, Corixidae, Baetide and Oligachatea were the most abundant taxa in farm ponds located in extensive agricultural systems dominated by livestock farming and irrigation. In grassland streams, Dolédec *et al.* (2006) found that densities of species of Plecoptera and Trichoptera decreased across land use gradient, while densities of species of Mollusca increased with increasing land use gradient. Similarly, Chironomidae abundance was significantly lower whereas Plecoptera and Amiphpoda abundances were significantly higher at reference sites compared to cornfield and pasture sites (Kyriakeas & Watzin, 2006). These changes in invertebrate community composition resulting from agricultural land use are similar to the current findings for both ponds and streams.

Nemouridae were the only Plecoptera family that was found in the study sites (Table 3.1), and they were almost absent in both types of ponds and in arable streams (Figure 3.3). Stoneflies have been reported as sensitive taxa and they are replaced by tolerant taxa when water quality is decreased (Myslinski & Ginsburg, 1977). In addition, Oligochaetes in this study were higher in arable streams and pastoral ponds. Oligochaetes are known to be pollution tolerant (Goodnight, 1973; Myslinski & Ginsburg, 1977) but they also tend to increase with increasing sediment (Goodnight, 1973; Kyriakeas & Watzin, 2006).

The hemipteran (Corixidae), which was found more in arable ponds in this study, is known to be tolerant to low concentrations of dissolved oxygen and likely to increase in eutrophic habitats (Solimini *et al.*, 2008). However, this does not appear to be the case in the current study where pastoral ponds had significantly lower concentrations of dissolved oxygen. Furthermore, arable ponds had higher abundance of Lymnaeidae whereas pastoral ponds contained greater abundance of Planorbidae. These snails are known to be tolerant to adverse conditions and able to live within a wide range of environmental variables (Myslinski & Ginsburg, 1977). Arable ponds were associated with high abundance of dragonflies (Coenagrionidae) in the current study. It has been reported that the abundances of certain species of dragonflies increase with slight to moderate nutrient enrichment resulting from urban wastewater treatment and agricultural pollution (Myslinski & Ginsburg, 1977).

Changes in aquatic invertebrate communities could be caused by high nutrient concentrations resulting from agricultural activities (Trigal *et al.*, 2007; Fuentes-Rodriguez *et al.*, 2013). High concentrations of phosphorus have been shown to affect aquatic macroinvertebrates in agricultural landscapes, resulting in lower invertebrate richness in highly degraded ponds (Trigal *et al.*, 2007), and a decrease in invertebrate diversity in farm ponds (Fuentes-Rodriguez *et al.*, 2013). Furthermore, high numbers of gastropods associated with high concentrations of nutrients (especially phosphorus) have been reported (Dolédec *et al.*, 2006; Piggott *et al.*, 2012; Czerniawski *et al.*, 2013). In addition, Piggott *et al.* (2012) found in a mesocosm study that nutrient enrichment (nitrogen and phosphorus) at intermediate and high levels caused an increase in abundances of Chironomidae and the Hydrobiidae sp. (*Potamopyrgus antipodarum*). However, high concentrations of nutrients do not necessarily make a stressful environment for freshwater invertebrates, and the negative effects of high nutrient levels on macroinvertebrates are expected only when these levels cause extensive algal growth (Dolédec *et al.*, 2006).

In this study, environmental variables are likely to influence invertebrate communities in ponds; pastoral ponds were associated with high concentrations of total phosphorus and low concentrations of dissolved oxygen. Further, not all pastoral ponds in this study are surrounded by fences or vegetation (as observed in PP2, PP4, PP5 and PP6), which allows livestock access and direct inputs of cattle faeces and urine. Consequently, higher loads of nutrients could be added to pond water. In contrast, arable and some pastoral streams had higher concentrations of total nitrogen, and most pastoral streams are bordered by either fences or riparian buffers. Thus, it appears that macroinvertebrate communities in arable streams were possibly influenced by physical modifications associated with high loads of nitrogen. However, the environmental variables are interdependent and interactive with each other and hence it is difficult to identify the main causes.

One potential limitation in this study is that invertebrates were identified to family level not species level. However, three invertebrate groups were identified to species level: Gastropoda because they were the main grazer group in study sites and their abundance declined in arable ponds and streams, Amphipoda because they were the main shredder family and play an important role in leaf decomposition, and Odonata because they have aesthetic value. The species composition for these groups did not differ between the two types of land use. Future work is to consider species identification for all macroinvertebrate groups.

3.5. Conclusion

This study demonstrated that although the invertebrate structure, feeding functional groups (FFGs) and community composition did not differ between arable and pastoral ponds, macroinvertebrates in ponds are affected by agricultural land use in general as indicated by high abundance of Diptera (up to 64% of the relative abundance in arable ponds) and a decrease in sensitive taxa. In streams, the total abundance, taxonomic richness, FFGs (mainly scrapers and shredders) and community composition were significantly different between arable and pastoral sites but diversity index was similar between the two types of land use.

The findings of this study suggested that arable farming showed a moderate negative effect on the invertebrate composition in streams, indicated by significantly high abundance of Oligochaeta and an almost total absence of stoneflies in arable sites. In ponds, arable and pastoral sites resulted in similar macroinvertebrate communities and most taxa were found in both land uses but they varied in their abundance. Any changes in the structure and/or community composition of invertebrates may have an effect on their functioning (Covich *et al.*, 2004; Dudgeon, 2010). The next chapter will investigate the effect of agricultural land use on ecosystem functioning (leaf litter decomposition).

Chapter 4: Leaf litter decomposition in agricultural ponds and streams located in different types of land use

4.1. Introduction

Leaf litter decomposition is an essential ecosystem process and represents the main energy source in terms of nutrients and organic carbon in aquatic and terrestrial ecosystems (Gessner *et al.*, 2010). Detritivore communities play an important role in litter decomposition. For example, the presence of terrestrial macroinvertebrates in a Hawaiian rainforest led to an increase in litter decomposition rates by 16.9% and an increase in nutrient release rates by 33.2% for nitrogen and 30.3% for manganese (Meyer *et al.*, 2011). In fact, it has been estimated that the impact of terrestrial macroinvertebrate activity on nitrogen release in a Hawaiian rainforest is greater than inputs from rain water, volcanic sources, dry deposition, nitrogen fixation and atmospheric dust (Meyer *et al.*, 2011). The litter decomposition process in aquatic and terrestrial ecosystems is similar (Gessner *et al.*, 2010). This chapter is focused on litter breakdown in small aquatic habitats located in agricultural landscapes.

Breakdown of leaf litter in freshwaters is controlled by chemical leaching, microbial decomposition (especially aquatic hyphomycetes), invertebrate feeding and physical abrasion (Webster & Benfield, 1986). Shredder invertebrates, like amphipods, stoneflies and caddisflies, play an important role in decomposition processes and nutrient cycling (Webster & Benfield, 1986; Graça, 2001), by converting coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) that other decomposers can utilize (Cummins, 1974; Wallace & Webster, 1996).

Many environmental factors influence leaf litter decomposition in freshwaters. In general, decomposition rates are faster at warmer temperatures (Webster & Benfield, 1986) because temperature primarily influences microbial decomposition (Irons *et al.*, 1994) whereas invertebrate feeding tends to be less affected (Webster & Benfield, 1986). Faster decomposition rates have been also observed in nutrient rich streams (Ferreira *et al.*, 2006; Bergfur *et al.*, 2007; Menéndez *et al.*, 2011; Tolkkinen *et al.*, 2013). For example, leaf decomposition rate was faster in the most eutrophic stream and lower in the less eutrophic stream in Spain (Menéndez *et al.*, 2011). However, other studies have not observed an effect of nutrient enrichment on decomposition (Baldy *et al.*, 2007; Fleituch, 2013; Pérez *et al.*, 2013). Moreover, slow decomposition rates have been observed in acidic streams, suggesting indirect effect of low pH on microorganisms and invertebrates (Webster & Benfield, 1986; Tolkkinen *et al.*, 2013).

In addition to environmental variables, anthropogenic disturbance and changes in land use may also affect leaf decomposition. Many studies have reported the impact of different anthropogenic land use (i.e. forest, agriculture and urban) on total leaf litter breakdown (Paul *et al.*, 2006; Torres & Ramirez, 2014; Voß *et al.*, 2015). While leaf breakdown rates were faster in agricultural and urban streams in the USA (Paul *et al.*, 2006), they were faster in forested streams in Brazil (Torres & Ramirez, 2014). Leaf decomposition in streams can be decreased as a result of anthropogenic-induced decreases in microbial conditioning and/or a reduction in shredder abundance or feeding activity (Webster & Benfield, 1986; Forrow & Maltby, 2000). A study conducted in streams located in different land use patterns (forested, agricultural, vinicultural and urban) found that while microbial breakdown rate was significantly affected by land use, there was no significant effect on invertebrate-mediated breakdown rate (Voß *et al.*, 2015).

A large body of research has focused on the effect of agriculture on leaf decomposition (Hagen *et al.*, 2006; Baldy *et al.*, 2007; Piscart *et al.*, 2009; Magbanua *et al.*, 2010; Piscart *et al.*, 2011; Jinggut *et al.*, 2012; Rasmussen *et al.*, 2012; Arroita *et al.*, 2013). For example, Piscart *et al.* (2009) found that agricultural intensity can strongly influence the activity of shredder invertebrates and decrease litter decomposition rates by up to 75% in the most intensive farming areas. Furthermore, investigating leaf litter breakdown in streams affected by different agricultural activities (traditional extensive farming, mainly livestock production, and vineyard cultivation), Piscart *et al.* (2011) found that the total leaf decomposition was strongly affected by changes in land use in the catchment. The total breakdown rates significantly decreased in areas with increasing vineyard pressure, but there was no significant difference between sites located within livestock farming areas (Piscart *et al.*, 2011). A study conducted in a tropical region in Malaysia comparing two agricultural practices found that streams in areas with traditional farming practices (slash and burn) had higher leaf decomposition rates and less impact on shredder communities than mechanized forest clearance, which had a negative impact on both shredder communities and leaf decomposition (Jinggut *et al.*, 2012). On the other hand, Magbanua *et al.* (2010) reported no significant differences in leaf breakdown rate between streams in areas of conventional, integrated or organic farming in southern New Zealand. Similarly, Hagen *et al.* (2006) found that leaf decomposition rates in southern Appalachian streams did not differ significantly along an agricultural land-use gradient. Most of these studies have focused on the intensity of agriculture on leaf decomposition (Hagen *et al.*, 2006; Magbanua *et al.*, 2010; Piscart *et al.*, 2011) but comparing two types of agricultural land use is limited.

The role of macroinvertebrates in leaf processing is crucial, and fast leaf litter decomposition have been reported in ecosystems with high invertebrate densities (Webster & Benfield, 1986). A positive relationship between leaf decomposition rates and shredder abundance has been observed in streams (Bergfur *et al.*, 2007; Piscart *et al.*, 2009; Menéndez *et al.*, 2011; Piscart *et al.*, 2011). For example, positive correlations have been reported between the abundance of the freshwater isopod *Asellus aquaticus* and leaf decomposition rates (Tolkkinen *et al.*, 2013), and between the abundance of freshwater amphipods (Gammaridae) and leaf litter decomposition in streams (Piscart *et al.*, 2009; Kunz *et al.*, 2010; Rasmussen *et al.*, 2012).

The feeding rate of *Gammarus pulex* has been used as a biomonitor of water quality in streams (Maltby *et al.*, 2002; Kunz *et al.*, 2010). Batista *et al.* (2012) stated that the use of feeding assay with a sublethal effect of containments could be a useful way to assess the potential toxicity of pollutants in freshwater. The feeding activity of *Gammarus* can be affected by biotic factors, for example, parasitism (Pascoe *et al.*, 1995), body size (Nilsson, 1974; Coulaud *et al.*, 2011), and population source (Maltby & Crane, 1994; Crane *et al.*, 1995); and also by abiotic factors such temperature (Maltby *et al.*, 2002; Coulaud *et al.*, 2011), pH (Naylor *et al.*, 1989) and food quality (Graça *et al.*, 1994). Reduction in the feeding rate of gammarids due to toxicant exposure can lead to reduced growth, size, fertility and survival of individuals (Anderson & Cummins, 1979; Maltby & Naylor, 1990).

A large number of laboratory studies have shown that the feeding rate of invertebrates can be inhibited by many different chemical stressors, such as unionized ammonia (Alonso & Camargo, 2004), metals (Blockwell *et al.*, 1998; Batista *et al.*, 2012) and pesticides (Blockwell *et al.*, 1998; Zubrod *et al.*, 2010; Agatz *et al.*, 2014). However, laboratory studies tend to focus on the effects of single stressors whereas, in natural systems, freshwater organisms are exposed to multiple stressors and may respond differently from in the laboratory (Duarte *et al.*, 2006; Fernandes *et al.*, 2009). In situ feeding assays that include the use of caged animals have the advantage of better control of stressors under natural conditions (Maltby & Burton, 2006). In situ assays have been used to investigate a range of contamination sources, including metalliferous effluents (Maltby & Crane, 1994), agriculture (Crane *et al.*, 1995), coal mine and industrial effluents (Maltby *et al.*, 2002), landfill leachates (Bloor & Banks, 2006) and pesticide spray drift (Maltby & Hills, 2008). Recently, Coulaud *et al.* (2011) found that the feeding rate of *Gammarus* was significantly inhibited in 37% of contaminated sites, suggesting that the feeding activity seemed to be sensitive to contaminants in a mix of anthropogenic activities (industrial, agricultural and mining).

It is widely recognised that leaf litter decomposition in streams is faster compared to lakes or ponds (Webster & Benfield, 1986). Many factors could explain the differences between streams and lakes, including the differences in leaching and physical abrasion (Witkamp & Frank, 1969), the current action in streams which could cause greater fragmentation and continually expose leaf surfaces to microorganisms (Hodkinson, 1975), and the less important role of invertebrate detritivores in lakes (Hodkinson, 1975; Gasith & Lawacz, 1976). Most research has focused on leaf litter decomposition in streams and rivers and decomposition in ponds is rarely considered.

The aim of this study is to quantify leaf decomposition processes in agricultural freshwater habitats located in different types of land use. This was achieved by measuring microbial and invertebrate-mediated leaf decomposition by deploying leaf bags of different mesh sizes in ponds and streams across an arable-pastoral gradient in agricultural catchments. Leaf material in coarse mesh bags is accessible to macroinvertebrates and mass loss is potentially a function of leaching and physical abrasion, microbial decomposition and invertebrate feeding. In contrast, fine mesh bags exclude (most) macroinvertebrates and hence mass loss is dependent on physical and microbial processes only (Bärlocher, 2005). In addition to decomposition studies, in situ feeding rate of *Gammarus pulex* was measured in stream sites. The objectives of this study were to: (1) compare decomposition rate (i.e. leaf mass loss) between arable and pastoral sites in ponds and streams; (2) assess how variation in the abundance of leaf-shredding invertebrates relates to decomposition processes; (3) explore the influence of land use on the in situ feeding rate of *Gammarus pulex* deployed in streams.

4.2. Methods

4.2.1. Study sites

Twenty four study sites (6 arable ponds, 6 pastoral ponds, 6 arable streams and 6 pastoral streams) in agricultural landscape in Eye Brook and Stonton Brook catchments in Leicestershire were selected (see Chapter 2 for a detailed description). Two field studies were conducted: leaf litter decomposition using leaf bags in 2013 and in situ feeding rate using cages containing *Gammarus pulex* in 2014.

4.2.2. Leaf decomposition

Leaf bags were used to quantify leaf decomposition in all pond and stream sites. Two hundred and forty leaf bags (25 cm × 20 cm) were made from two types of mesh: 120 bags were made using coarse plastic mesh (pore size: 7 mm × 5 mm) and 120 bags were made using fine nylon mesh (pore size: 600 µm × 400 µm). Coarse mesh bags allowed macroinvertebrates to pass through, but fine mesh bags did not.

Alder leaves (*Alnus glutinosa*) were collected after abscission and before leaf fall in October 2011 from Stainborough Dyke (National Grid Reference: SE 323 040) and Shalesmoor (National Grid Reference: SK 340 876), United Kingdom. Leaves were air dried at room temperature for a week then stored at room temperature until use. Five grams of air dried leaves were placed in each bag. Ten bags were deployed per site, five coarse mesh and five fine mesh bags. Coarse and fine mesh bag were paired and pairs of bags were attached by fishing line to a wooden stake or a tree. All leaf bags were deployed in February 2013.

After seven weeks, all leaf bags were collected, sealed in individual plastic bags, transferred to the laboratory in a cool box and stored in a freezer at −18°C until processing. After defrosting, macroinvertebrates were removed from leaves, sorted and placed in 70% industrial methylated spirits (IMS) for later identification. The remaining leaf material in each mesh bag was rinsed gently through a 500 µm sieve to remove sediment particles, oven-dried at 60°C for three days and weighed. In order to convert oven-dried leaf mass (M_d, g) to air-dried leaf mass (M_a, g), an air-dried mass to oven-dried mass relationship was generated from fifty sets of leaf samples using linear regression ($r^2 = 0.99$) (Equation 4.1):

$$M_a = 0.01 + 1.06\, M_d \qquad \text{Equation 4.1}$$

4.2.3. In situ feeding rate

In situ field experiment followed the methods described in Maltby *et al.* (1990) and Crane and Maltby (1991). Cages contained *Gammaus pulex* were deployed in 12 streams in April 2014 to determine in situ feeding rate. Gammaridae were very abundant in the study streams but rare or absent from many study ponds (Chapter 3). Consequently, in situ assays were only performed in streams.

4.2.3.1. Fungus culture preparation

Cladosporium herbarum (Reference No. 049630) was obtained from CABI (Centre for Agriculture and Biosciences International) and the stock culture was maintained in an incubator at 20°C. Prior to use in experiments, *C. herbarum* was grown on 3% malt extract agar, which was prepared by adding 30 grams malt extract, 15 grams agar and 5 grams mycological peptone to 1 litre distilled water. Cultures were incubated at 23°C for 7-12 days, after which *C. herbarum* was sub-cultured into malt extract broth, which was made by dissolving 30 grams malt extract and 5 grams mycological peptone in 1 litre distilled water, and then incubated at 23°C for 10-15 days.

4.2.3.2. Preparing leaf discs

Leaf discs were prepared using the method described in Naylor *et al.* (1989). A total of 1800 leaf discs were prepared from alder leaves (*A. glutinosa*) collected in October 2012 from Endcliffe Park (National Grid Reference: SK 323 858). Air dried alder leaves were rehydrated with distilled water and cut into discs (1.6 cm diameter) using a cork borer and avoiding main veins. Groups of 120 leaf discs were each placed in a 500-ml conical flask containing 300 ml enriched water (Naylor *et al.*, 1989) and autoclaved at 121°C for 20 minutes. Autoclaved leaf discs were inoculated with the fungus *C. herbarum* from a culture on malt extract broth by adding six discs (1.6 cm diameter) of the fungus to each flask. The leaf discs were then incubated on an orbital shaker for 10 days at room temperature (20 – 22 °C). All leaf discs were then removed from the flasks, dried at 60°C in an oven for 2 days and stored in an airtight container until use. Before field deployment, dried leaf discs were allocated to 360 sets of five discs and weighed.

4.2.3.3. Animal collection and maintenance

Adult male *Gammarus pulex* (Crustacea, Amphipoda) were collected from Crags Stream in Clowne, Derbyshire, United Kingdom (National Grid Reference: SK 497 744) using standard kick-net sampling. The contents of each sample were placed in a sorting tray and *G. pulex* were moved to a bucket containing stream water using a small plastic sieve. This process was continued until the required number of animals (i.e. 300 individuals) had been collected. The animals were transported to the laboratory, allocated to four 2-L plastic containers containing stream water (approx. 75 animals/container) and placed in a controlled room at 15 ± 0.5 °C. After 24 hours, stream water was gradually replaced by aerated artificial pond water (APW) (Naylor et al, 1989) and animals were fed with alder leaves conditioned with the fungus *C. herbarum*. All animals were maintained for a week at 15 ± 0.5 °C and 12 h light: 12 h dark photoperiod before being used in the field deployment.

4.2.3.4. Field deployment

Thirty polyvinyl chloride cages (length: 5cm, diameter: 5cm) were deployed at each stream site: 25 cages containing an individual animal with leaf discs and 5 cages containing leaf discs only. A set of five leaf discs was placed in all cages and rehydrated with tap water for 12 hours before deployment. In the field, 25 adult male *G. pulex* were placed in cages (1 per cage) and cage ends were capped with 1 mm^2 mesh. Cages were randomly allocated mesh baskets (10 cages per basket) and placed with cage bores parallel to the direction of water flow. Three baskets per site were secured to the stream bed using tent pegs and fishing line. After 6 days, all cages were collected and dead animals were counted. All surviving animals were removed, oven dried at 60°C for four days and weighed. Remaining leaf discs in each cage were removed, gently washed with tap water and oven dried at 60°C for four days before weighing.

4.2.3.5. Water chemistry

Physiochemical characteristics of pond and stream water were measured at the same time as animal cages deployment in April 2014. In the field, measurements of water temperature (°C), pH, dissolved oxygen (mg/l) and conductivity (µs/cm^2) were made using a hand held meter (YSI Professional Plus). In addition, three 300 ml water samples were collected from each site, transported to the laboratory in a cool box, stored in a freezer at (−18 °C) and then analysed as soon as possible. Each water sample was filtered (Minisart$^{®}$ high flow syringe filters) and analysed for nitrate, nitrite, ammonia, phosphate and alkalinity ($CaCO_3$) concentrations using a Palintest Interface Photometer 7500.

4.2.4. Data analysis

Leaf decomposition was expressed as leaf mass loss over the seven week deployment period and calculated by subtracting the final leaf mass from the initial leaf mass (Equation 4.2):

$$\text{Leaf Mass Loss (g)} = M_i - M_f \qquad \text{Equation 4.2}$$

where M_i was the dry leaf mass pre-deployment (air-dried, g) and M_f was the dry leaf mass post-deployment (air-dried, g).

In order to estimate decomposition rates, an exponential breakdown coefficient (k) was calculated using Equation 4.3 (Bärlocher, 2005):

$$M_t = M_0 \,.\, e^{-kt} \qquad \text{Equation 4.3}$$

where M_t was the dry leaf mass (g) at time t (days), M_0 was the dry leaf mass (g) at time 0 and k was exponential decay coefficient.

Leaf mass loss and breakdown coefficients were calculated for each leaf bag and averaged for each leaf bag type (i.e. coarse, fine) deployed at each site. Two-way ANOVAs were used to analyse the effect of land use (arable / pastoral) and bag type (coarse / fine) on the leaf mass loss and breakdown coefficient in ponds and streams. Normality and homogeneity of variance were checked using Shapiro-Wilk and Levene's tests and data were natural log transformed if needed.

The abundance of invertebrates associated with leaf decomposition (shredders and Chironomidae) were averaged for each site. Two-sample *t*-tests were used to compare shredder abundance, richness and diversity (measured as Shannon index) and Chironomidae abundance between the two types of land use. Shredder abundance, shredder diversity and Chironomidae abundance were ln(x+1) transformed.

In order to explore the role of invertebrates in the leaf breakdown process, the amount of leaf processing caused by shredders was estimated from the difference in leaf loss between coarse and fine mesh bags. Linear regressions were used to explore the relationships between: total leaf mass loss and shredder abundance in coarse mesh bags; shredder-mediated leaf mass loss and shredder abundance in coarse mesh bags; total leaf mass loss and Chrionomidae abundance in coarse mesh bags; and microbial leaf mass loss and Chrionomidae abundance in fine mesh bags.

In order to detect which shredder families accounted for significant variation in decomposition, Pearson correlations were used to investigate the associations between total and shredder-mediated leaf mass loss and the abundances of each family of shredders. All shredder families were ln (x+1) transformed before the analysis.

The feeding rate of individual *G. pulex* (*FR*, mg dry food weight/ mg dry animal weight/ day) was calculated using Equation 4.4 (Maltby *et al.*, 2002):

$$FR = \frac{(L_i \times C) - L_f}{W \times t} \qquad \text{Equation 4.4}$$

where L_i is initial dry weight of leaf discs (mg), L_f is final dry weight of leaf discs (mg), W is dry weight of the animal (mg), t is experiment period (6 days) and C is correction factor for non-consumptive mass loss, given by the mean of the quotient of final to initial dry weight of control leaf discs. The unexpected presence of small gammarids in control cages meant that it was not possible to calculate a reliable correction factor and therefore uncorrected feeding rates were calculated. The average feeding rate of deployed individuals was calculated for each site, and two-sample *t*-test was used to assess the effect of land use on *G. pulex* feeding rate. Pearson correlations were used to investigate the associations between the physiochemical characteristics of stream water (pH, conductivity, alkalinity, ammonia, phosphate, nitrate and nitrite) and the feeding rate. Water temperature and dissolved oxygen were excluded from the analysis and natural logarithm transformations were used where necessary.

All statistical analyses were performed using R (R Core Team, 2013), and the package ggplot2 (Wickham, 2009) was used for producing figures.

4.3. Results

4.3.1. Leaf decomposition

Mean leaf mass loss in coarse mesh bags deployed in ponds ranged from 1.8 to 3.5 g for arable sites, and from 1.5 to 2.2 g for pastoral sites. Leaf mass loss in fine mesh bags ranged from 1.8 to 2.3 g for arable sites, and from 1.5 to 2.2 g for pastoral sites. Leaf mass loss (Figure 4.1a) and breakdown coefficient (Figure 4.2a) were significantly higher in arable ponds than pastoral ponds ($p < 0.05$, Table 4.1). There was no significant effect of leaf bag type (i.e. coarse mesh or fine mesh) or significant interaction between land use and bag type on either leaf loss or breakdown coefficient (Table 4.1).

There was no significant effect of land use on either the mass loss or the breakdown coefficient of leaf material deployed in streams (Table 4.1). Leaf mass loss in coarse mesh bags was lower in arable sites (range 2.1 to 4.6 g) than in pastoral sites (range 2.6 to 4.4 g), but leaf mass loss in fine mesh bags was similar for both land use types (arable: 2.2 to 2.5 g, pastoral: 2.0 to 2.5 g).

Leaf mass loss (Figure 4.1b) and breakdown coefficient (Figure 4.2b) were both significantly higher in coarse mesh bags than in fine mesh bags ($p < 0.001$), but there was no significant interaction between land use and mesh bag types on leaf mass loss (Table 4.1).

Table 4.1 Two-way ANOVAs the effect of land use and bag type on leaf mass loss (g) and breakdown coefficient (k) in ponds and streams. Significant differences are highlighted in bold ($p < 0.05$).

Factor		**Leaf Mass Loss (g)**		**Breakdown coefficient (k)**	
	df	F	*p*	F	*p*
In ponds					
Land use	1	7.18	**< 0.05**	6.14	**< 0.05**
Bag type	1	0.78	0.39	0.89	0.36
Land use x Bag type	1	0.27	0.61	0.42	0.53
Residuals	20				
In streams					
Land use	1	0.60	0.46	0.56	0.46
Bag type	1	17.3	**< 0.001**	17.0	**< 0.001**
Land use x Bag type	1	1.15	0.30	0.86	0.37
Residuals	20				

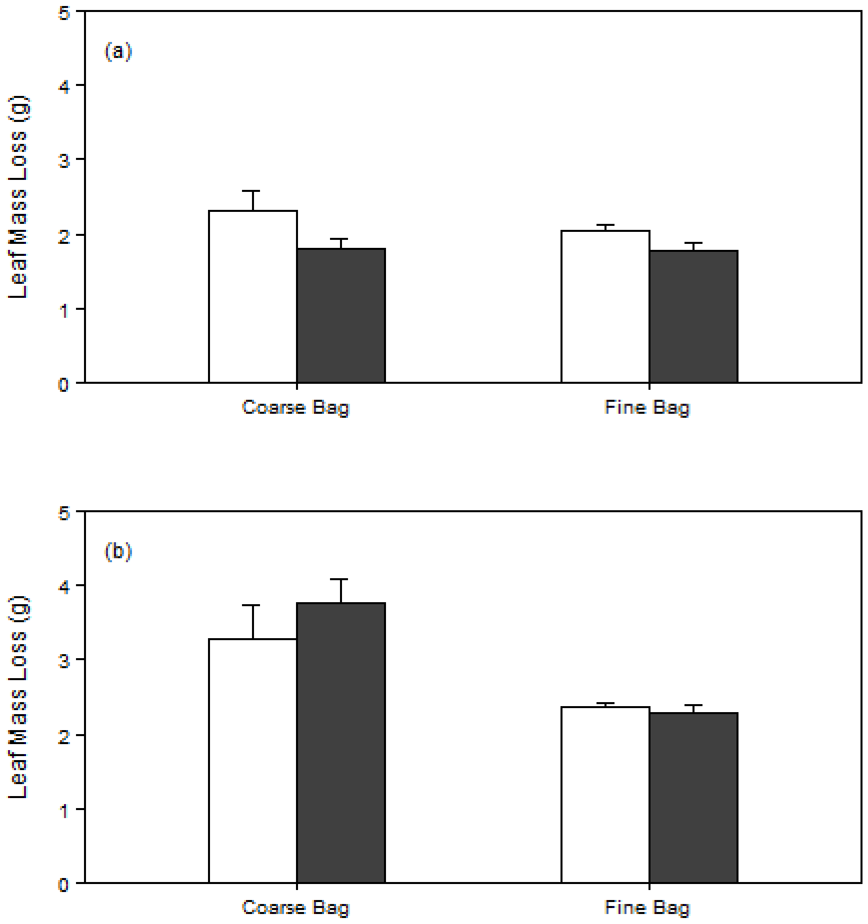

Figure 4.1 Mean (+ SE) leaf mass loss (g) in coarse and fine bags in arable (white bars) and pastoral (black bars) in (a) ponds and (b) streams.

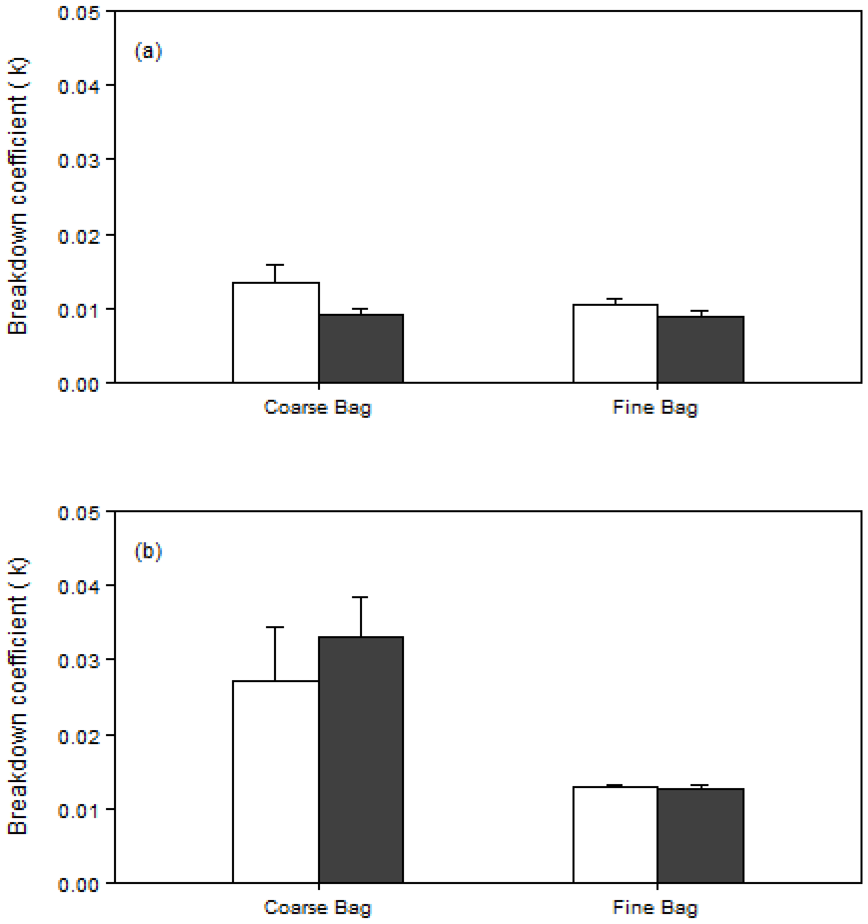

Figure 4.2 Mean (+ SE) breakdown coefficient (*k*) in coarse and fine bags in arable (white bars) and pastoral (black bars) in (a) ponds and (b) streams.

4.3.2. Macroinvertebrates in leaf bags

A total of 14601 macroinvertebrate individuals from 37 families were present in coarse leaf bags deployed in ponds and streams. The relative abundance of invertebrate families that constitute more than 1% of the overall abundance is presented in Table 4.2. Chironomidae were the main dominant family found in coarse leaf bags and accounted for 71.1% and 72.7% of individuals in ponds and streams, respectively (Table 4.2). Shredder families accounted for only 9.5% of individuals in ponds while they accounted for 20.3% of individuals in streams (Table 4.2). In contrast, non-shredder families excluding Chrionomidae accounted for 19.4% of individuals in ponds whereas they accounted for only 7% of individuals in streams (Table 4.2).

The unexpected presence of invertebrates in fine leaf bags was detected (Table 4.3). The dipteran families Chironomidae and Ceratopogonidae were the main families that were present in fine bags and they accounted for 95.8% of individuals in ponds whereas shredder families only accounted for 2.9% of individuals in ponds (Table 4.3). In streams, Chironomidae accounted for 82.6% of individuals whereas Gammaridae accounted for 14.1% of individuals (Table 4.3).

The abundance, richness and diversity (Shannon index) of shredders found in coarse leaf bags deployed in both ponds and streams are presented in Figure 4.3. Although there was no significant difference in shredder abundance between arable and pastoral ponds (two-sample *t*-test: $t_{10} = 0.24$, $p > 0.05$; Figure 4.3a), shredder diversity was significantly higher in arable than pastoral ponds and shredder richness showed marginal significance (two-sample *t*-test: $t_{10} = 2.67$, $p < 0.05$; $t_{10} = 1.93$, $p = 0.08$, respectively). The opposite pattern was found in streams, where shredder abundance was significantly higher in pastoral than arable sites (two-sample *t*-test: $t_{10} = 2.86$, $p < 0.05$; Figure 4.3b), but shredder richness and diversity did not differ significantly between the two land use types (two-sample *t*-test: all $t_{10} < 1.22$, $p > 0.05$). In addition, there were no significant differences in Chironomidae abundances found in coarse or fine mesh bags between arable and pastoral sites in either ponds or streams (two-sample *t*-test: all $t_{10} < 1.72$, $p > 0.05$).

Table 4.2 The relative abundance (%) of invertebrate families found in **coarse** leaf bags deployed in ponds and streams.

Habitat	**Invertebrate taxa**	**Invertebrate family**	**Relative abundance (%)**	**Group**
Ponds	Diptera	Chironomidae	71.1	Non-shredders
	Gastropoda	Planorbidae	11.2	Non-shredders
	Isopoda	Asellidae	8.0	Shredders
	Diptera	Ceratopogonidae	4.9	Non-shredders
	Rhynchobdellida	Glossiphoniidae	1.1	Non-shredders
	Other shredder taxa		1.5	Shredders
	Other non-shredder taxa		2.2	Non-shredders
Streams	Diptera	Chironomidae	72.7	Non-shredders
	Amphipoda	Gammaridae	15.6	Shredders
	Coleoptera	Scirtidae Larvae	3.9	Non-shredders
	Plecoptera	Nemouridae	2.5	Shredders
	Trichoptera	Limnephilidae	1.4	Shredders
	Diptera	Simuliidae	1.3	Non-shredders
	Other shredder taxa		0.8	Shredders
	Other non-shredder taxa		1.8	Non-shredders

Table 4.3 The relative abundance (%) of invertebrate families found in **fine** leaf bags deployed in ponds and streams.

Habitat	**Invertebrate taxa**	**Invertebrate family**	**Relative abundance (%)**	**Group**
Ponds	Diptera	Chironomidae	90.9	Non-shredders
	Diptera	Ceratopogonidae	4.9	Non-shredders
	Isopoda	Asellidae	1.5	Shredders
	Amphipoda	Crangonyctidae	1.3	Shredders
	Other shredder taxa		0.1	Shredders
	Other non-shredder taxa		1.3	Non-shredders
Streams	Diptera	Chironomidae	82.6	Non-shredders
	Amphipoda	Gammaridae	14.1	Shredders
	Other shredder taxa		1.5	Shredders
	Other non-shredder taxa		1.8	Non-shredders

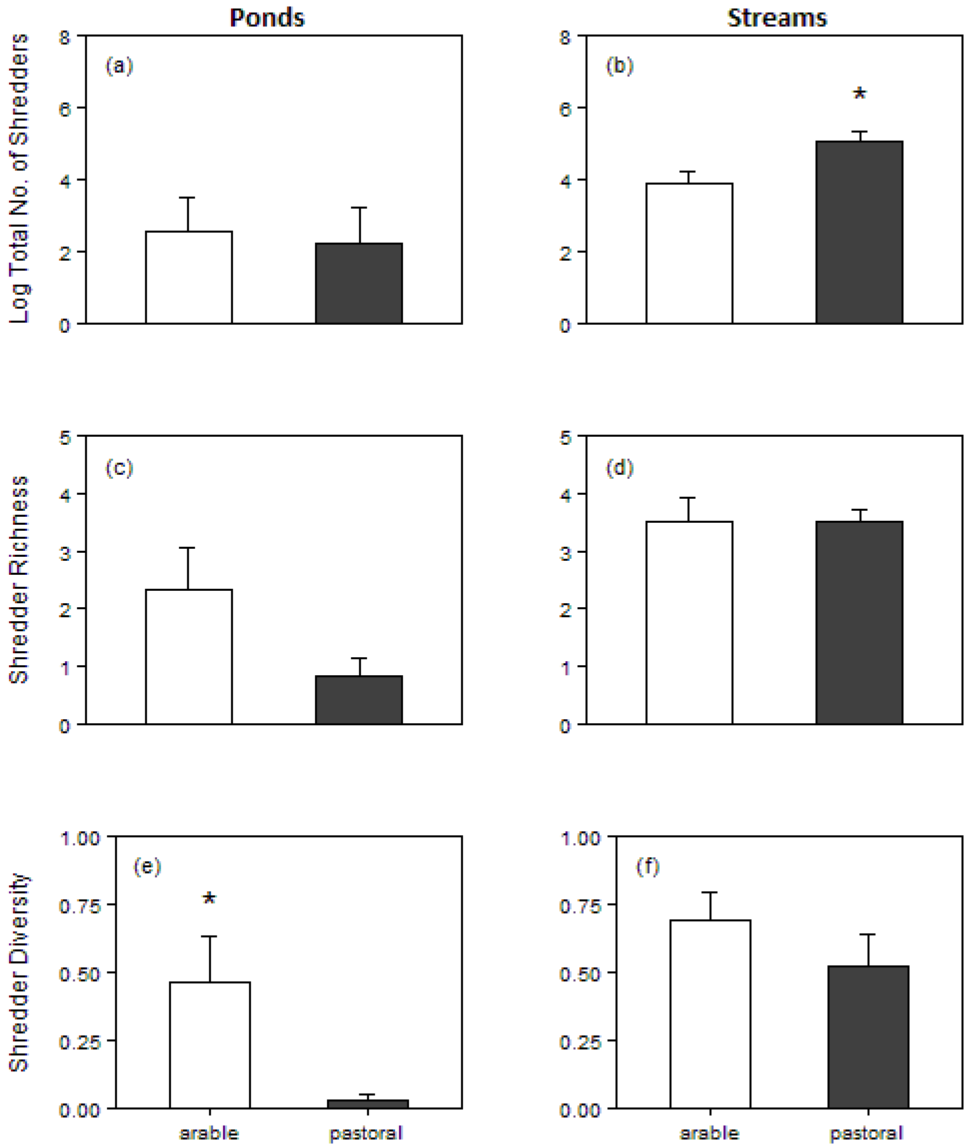

Figure 4.3 Mean (+ SE) Shredder abundance (a, b), Shredder richness (c, d) and Shredder diversity 'Shannon index' (e, f) found in coarse mesh bags in arable (white bars) and pastoral (black bars) in ponds on the left side and streams on the right side. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

Comparison of shredder invertebrates present in coarse leaf bags with those in benthic communities (Chapter 3) revealed similar patterns. The relative abundances of shredder families present in benthic communities and in coarse bags deployed in ponds and streams are presented in Table 4.4. All families found in coarse leaf bags were present in benthic samples. The most abundant families in benthic samples in ponds were Asellidae, Crangonyctidae, Gammaridae, Helophoridae and Limnephilidae whereas Asellidae and Crangonyctidae were the most abundant in leaf bags deployed in ponds (Table 4.4). Gammaridae, Nemouridae, Limnephilidae were the most abundant families that were found in both benthic samples and bags in streams (Table 4.4).

Shredder abundance in benthic samples was significantly higher in pastoral streams than arable streams whereas no significant difference between land uses was found in ponds (Section 3.3.3., Figure 3.6). The richness and diversity of shredders in benthic samples were higher in arable ponds than pastoral ponds; however, these differences between land uses were non-significant (two-sample *t*-test; richness: $t_{10} = 0.95$, $p > 0.05$; diversity: $t_{10} = 1.71$, $p > 0.05$). There were no significant differences between land uses in the richness or diversity of shredders in benthic stream samples (two-sample *t*-test; richness: $t_{10} = 0.82$, $p > 0.05$; diversity: $t_{10} = 0.65$, $p > 0.05$). Further, there were no significant differences in Chironomidae abundances in benthic samples in either ponds or streams (two-sample *t*-test; ponds: $t_{10} = 1.86$, $p > 0.05$; streams: $t_{10} = 1.19$, $p > 0.05$).

Table 4.4 The relative abundance (RA, %) of shredder families present in benthic communities and in coarse bags deployed in ponds and streams.

Habitat	Shredder families	RA (%) in benthic community	RA (%) in leaf bags
Ponds	Asellidae	0.6	8.0
	Gammaridae	1.1	0.3
	Crangonyctidae	0.9	0.7
	Nemouridae	0.03	−
	Curculionidae	0.1	−
	Haliplidae	0.4	0.2
	Helophoridae	2.0	−
	Leptoceridae	0.4	0.2
	Limnephilidae	0.8	0.2
	Limoniidae	0.02	0.01
	Tipulidae	0.1	−
	Lepidoptera	0.1	−
Total relative abundance (%)		**6.55**	**9.61**
Streams	Asellidae	0.03	−
	Gammaridae	31.1	15.6
	Nemouridae	1.9	2.5
	Curculionidae	0.04	−
	Helophoridae	0.09	0.1
	Lepidostomatidae	0.03	−
	Leptoceridae	0.003	−
	Limnephilidae	2.1	1.4
	Limoniidae	0.2	0.6
	Tipulidae	0.02	0.1
	Lepidoptera	0.02	−
Total relative abundance (%)		**35.5**	**20.3**

The relationships between leaf mass loss and the abundance of invertebrates in leaf bags were explored using simple linear regressions. There was a significant positive relationship between total leaf mass loss in coarse mesh bags and the abundance of shredders in those bags for ponds ($F_{1,10}$ = 8.65, $p < 0.05$, r^2 = 0.46; Figure 4.4a) and a marginally non-significant positive relationship for streams ($F_{1, 10}$ = 3.51, p = 0.09, r^2 = 0.26; Figure 4.4b). Comparing within land use type, however, this relationship was only significant for arable ponds ($F_{1, 4}$ = 8.65, $p < 0.05$, r^2 = 0.68). There was also a positive relationship between shredder-mediated leaf mass (i.e. difference between leaf mass loss in coarse and fine mesh bags) and the abundance of shredders in coarse mesh bags, which was marginally non-significant in both ponds ($F_{1, 10}$ = 3.70, p = 0.08, r^2 = 0.27; Figure 4.4c) and streams ($F_{1, 10}$ = 3.53, p = 0.09, r^2 = 0.26; Figure 4.4d). However, no significant relationships were detected when data were analysed for each land use separately. Further, there were no significant relationships between shredder richness or shredder diversity and shredder-mediated leaf loss in either ponds or streams (all: $F_{1, 10} < 3.4$, $p > 0.05$).

There were no significant relationships between total leaf mass loss in coarse mesh bags and the abundance of Chironomidae in those bags in either ponds or streams (Figure 4.5a and 4.5b). However, when compared within land use type, these relationships were positive and marginally non-significant for pastoral ponds ($F_{1, 4}$ = 6.72, p = 0.06, r^2 = 0.63) and almost significant for arable streams ($F_{1, 4}$ = 7.60, p = 0.051, r^2 = 0.66). In addition, microbial leaf mass loss in fine mesh bags was positively related to the abundance of Chironomidae in those bags. This relationship was significant for bags deployed in ponds ($F_{1, 10}$ = 14.19, $p < 0.01$, r^2 = 0.59, Figure 4.5c) and marginally non-significant for bags deployed in streams ($F_{1, 10}$ = 3.8, p = 0.08, r^2 = 0.28; Figure 4.5d). However, when compared within land use type, these relationships were only significant for arable ponds ($F_{1, 4}$ = 12.9, $p < 0.05$, r^2 = 0.76) and marginally non-significant for pastoral streams ($F_{1, 4}$ = 6.65, p = 0.06, r^2 = 0.62).

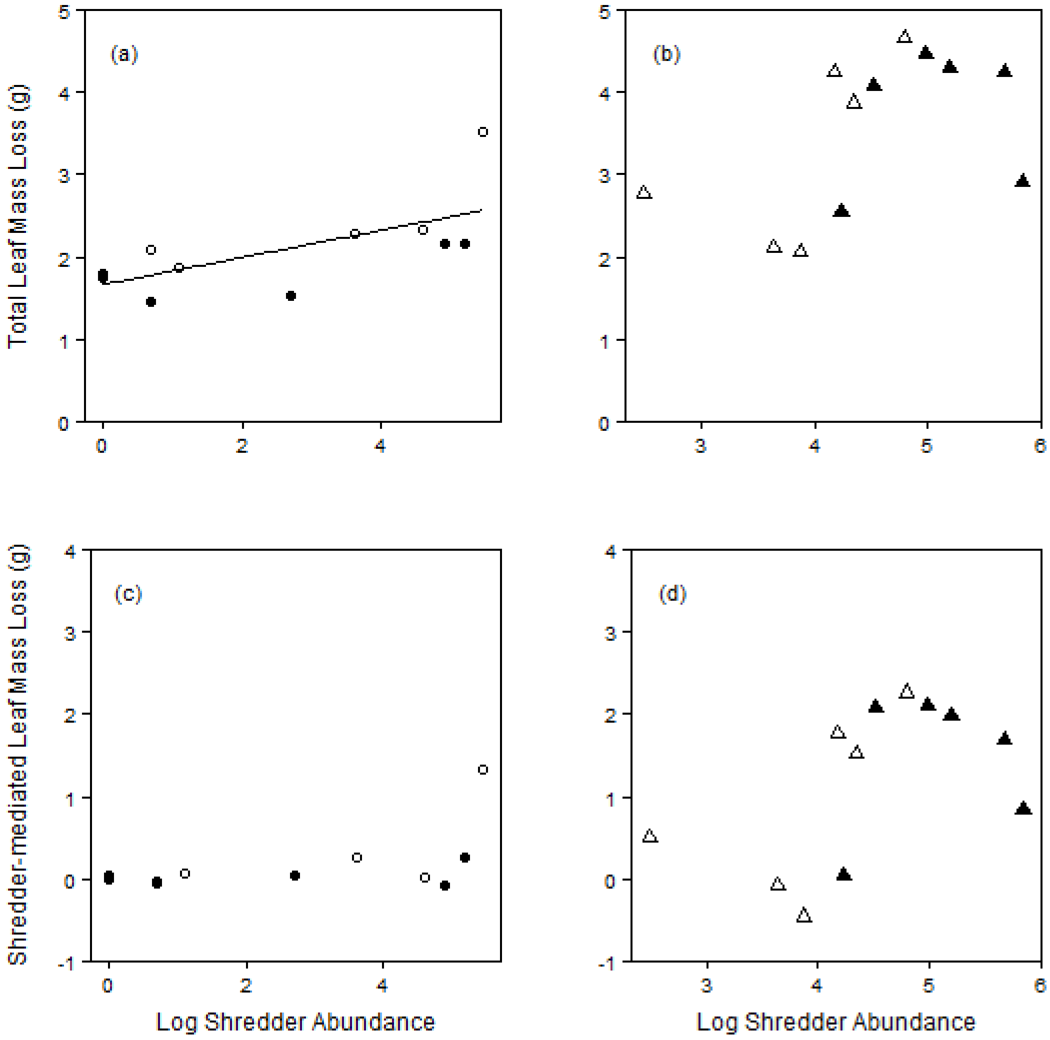

Figure 4.4 Relationships between the abundance of shredders in coarse leaf bags and total leaf mass loss in coarse mesh bags (a, b) and shredder-mediated leaf mass loss (c, d) in ponds (a, c) and streams (b, d). Open circles and triangles indicate arable sites and closed circles and triangles indicate pastoral sites.

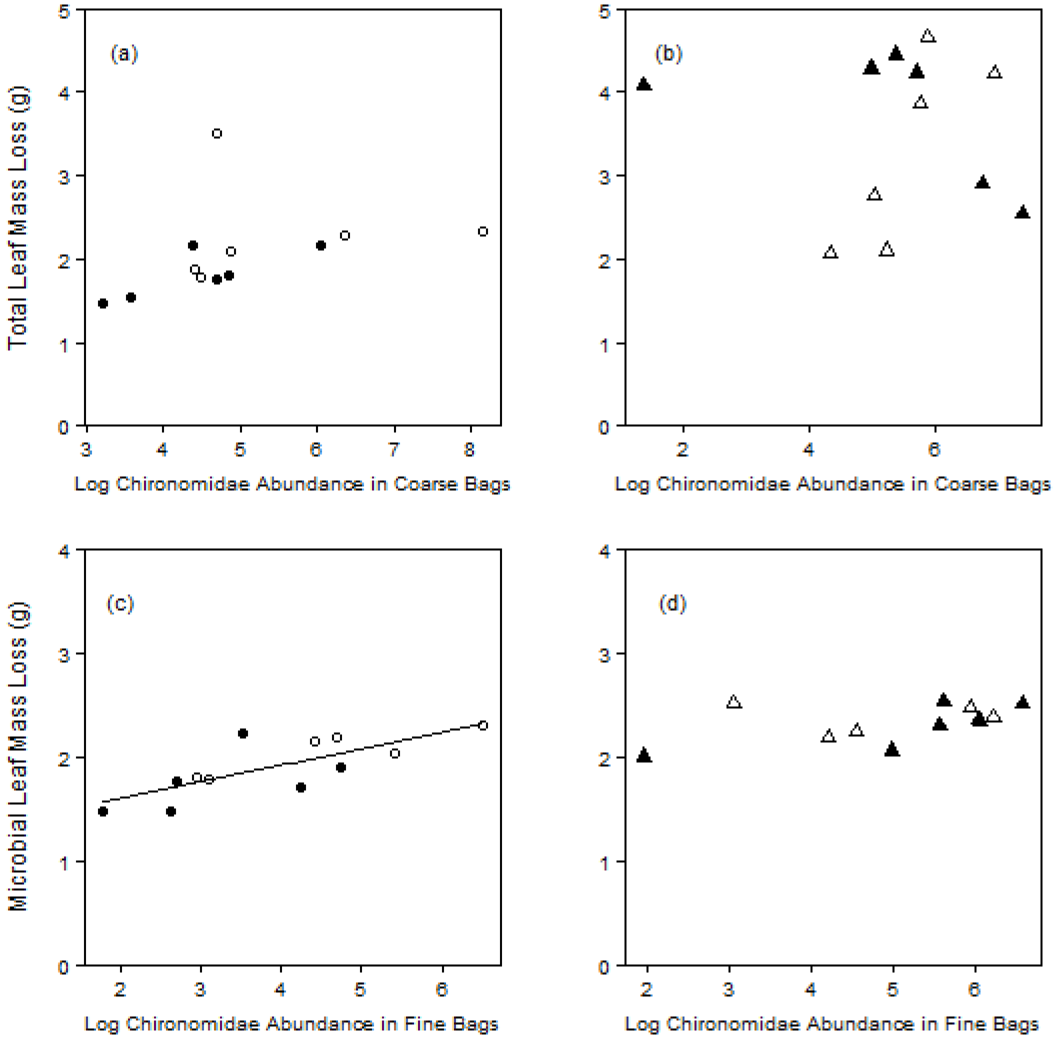

Figure 4.5 Relationships between the abundance of Chironomidae in coarse leaf bags and total leaf mass loss in coarse mesh bags (a, b) the abundance of Chironomidae in fine leaf bags and microbial leaf mass loss in fine mesh bags (c, d) in ponds (a, c) and streams (b, d). Open circles and triangles indicate arable sites and closed circles and triangles indicate pastoral sites.

Correlation coefficients for the relationship between total and shredder-mediated leaf mass loss and the abundance of individual shredder families are presented in Table 4.3. Although the isopod Asellidae was the most abundant shredder family in ponds (accounting for 8% of the overall invertebrate abundance found in coarse bags deployed in ponds), there was no significant correlation between Asellidae abundance and shredder-mediated mass loss (Table 4.5). There was, however, significant correlation between Asellidae abundance and total mass loss (Table 4.5). In addition, there were significant correlations between the abundance of the amphipod Crangonyctidae and the caddisfly Limnephilidae and both total and shredder-mediated mass loss in ponds (Table 4.5). Although Gammaridae was the most abundant shredder family in streams (accounting for 15.6% of the overall invertebrate abundance found in coarse bags deployed in streams), significant positive correlations with shredder-mediated mass loss were only detected for Limnephilidae (Table 4.5).

Table 4.5 Pearson correlations (r) between total and shredder-mediated leaf mass loss (LML) and abundances of shredder families; NS: not significant (*p* > 0.05).

Habitat	**Shredder Order**	**Shredder Family**	**Total LML**		**Shredder-mediated LML**	
			r	*p*	r	*p*
Ponds	Isopoda	Asellidae	0.59	< 0.05	0.45	NS
	Amphipoda	Crangonyctidae	0.80	< 0.01	0.87	< 0.001
	Amphipoda	Gammaridae	0.18	NS	0.15	NS
	Trichoptera	Limnephilidae	0.67	< 0.05	0.70	< 0.05
Streams	Amphipoda	Gammaridae	0.36	NS	0.38	NS
	Plecoptera	Nemouridae	0.39	NS	0.40	NS
	Trichoptera	Limnephilidae	0.54	= 0.07	0.57	= 0.054

4.3.3. Water chemistry

Water chemistry data for pond and stream sites in 2014 are presented in Table 4.6. Arable ponds had significantly higher concentrations of nitrate (two-sample *t*-test: $t_{10} = 3.86$, $p < 0.01$) and nitrite (two-sample *t*-test: $t_{10} = 3.06$, $p < 0.05$) than pastoral ponds. None of the other comparisons was statistically significant, although pastoral ponds had higher concentrations of ammonia (two-sample *t*-test: $t_{10} = 1.08$, $p > 0.05$) and lower concentrations of dissolved oxygen (two-sample *t*-test: $t_{10} = 1.86$, $p > 0.05$) than arable ponds.

For streams, arable sites had higher concentrations of phosphate, ammonia, nitrate and nitrite than pastoral sites; however, these differences were only statistically significant for nitrite (two-sample *t*-test: $t_{10} = 4.15$, $p < 0.01$).

Table 4.6 Mean ± SE and two-sample *t*-test physiochemical characteristics of water for the study sites. Water chemistry data collected across ponds and streams in **2014**. Significant differences are highlighted in bold ($p < 0.05$).

Habitat	Land use	Temperature (°C)	pH	Conductivity (µS/cm^2)	Dissolved Oxygen (mg/l)	Alkalinity (mg/l CaCO$_3$)	Phosphate (mg/l PO$_4$)	Ammonia (mg/l N)	Nitrate (mg/l N)	Nitrite (mg/l N)
Ponds	**Arable**	11.58 ± 0.40	7.44 ± 0.33	447.6 ± 77.74	10.76 ± 2.51	98.06 ± 18.89	0.088 ± 0.03	0.032 ± 0.01	5.422 ± 2.10	0.020 ± 0.01
	Pastoral	10.07 ± 0.38	6.98 ± 0.16	441.6 ± 78.94	5.12 ± 1.69	122.78 ± 11.69	0.091 ± 0.02	0.062 ± 0.02	0.049 ± 0.01	0.002 ± 0.00
Two-sample *t*-test *df = 10*		t = 2.75 **$p < 0.05$**	t =1.25 NSD	t = 0.05 NSD	t = 1.86 NSD	t = 1.11 NSD	t = 0.08 NSD	t = 1.08 NSD	t = 3.86 **$p < 0.01$**	t = 3.06 **$p < 0.05$**
Streams	**Arable**	9.65 ± 0.30	8.14 ± 0.10	476.2 ± 29.25	10.54 ± 0.33	155.28 ± 11.61	0.208 ± 0.14	0.040 ± 0.01	7.550 ± 2.25	0.025 ± 0.01
	Pastoral	10.52 ± 0.39	8.28 ± 0.10	554.9 ± 24.57	10.89 ± 0.22	147.78 ± 10.50	0.050 ± 0.01	0.021 ± 0.00	5.652 ± 2.63	0.005 ± 0.00
Two-sample *t*-test *df = 10*		t = 1.77 NSD	t = 0.95 NSD	t = 2.06 NSD	t = 0.89 NSD	t = 0.48 NSD	t = 0.81 NSD	t = 1.88 NSD	t = 0.55 NSD	t = 4.15 **$p < 0.01$**

NSD = no significant difference ($p > 0.05$).

4.3.4. In situ feeding rate

Survival of *Gammarus pulex* deployed for 6 days was > 98% at both arable and pastoral sites. The mean feeding rate of *G. pulex* deployed in pastoral streams was higher than that of animals deployed in arable streams, mean values being 0.27 mg food / mg dried animal / day and 0.21 mg food / mg dried animal / day, respectively. However, this difference was not statistically significant (two-sample *t*-test: $t_{10} = 1.21$, $p > 0.05$, Figure 4.6).

The correlations between the feeding rate of *G. pulex* and water chemistry parameters are presented in Table 4.7. Feeding rate was positively correlated with nitrate concentration ($r = 0.69$, $p = 0.013$) but negatively correlated with ammonia concentration ($r = -0.67$, $p = 0.016$). There were no significant correlations between the feeding rate and other environmental variables (all: $p > 0.05$, Table 4.7).

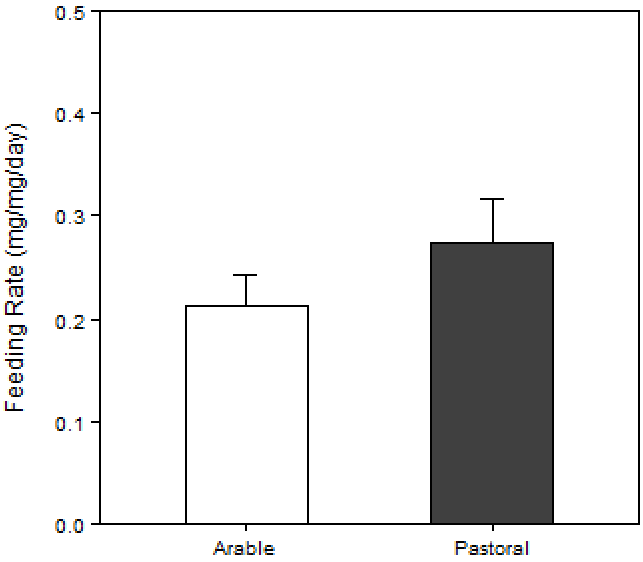

Figure 4.6 Mean (+ SE) feeding rate of *G. pulex* in streams in arable (white bars) and pastoral (black bars) sites.

Table 4.7 Pearson correlations between feeding rate of *G. pulex* and the physiochemical characteristics of stream water. Significant correlations are in bold ($p < 0.05$).

Variable	**Correlation coefficient**	***p***
pH	-0.09	0.77
Conductivity (µg/cm^2)	0.44	0.16
Alkalinity (mg/l $CaCO_3$)	0.20	0.54
Phosphate (mg/l PO_4)	-0.26	0.42
Ammonia (mg/l N)	**-0.67**	**0.016**
Nitrate (mg/l N)	**0.69**	**0.013**
Nitrite (mg/l N)	-0.41	0.19

4.4. Discussion

The objectives of this study were to compare the effects of different types of agricultural land use on leaf litter decomposition in ponds and streams, to assess how variation in the abundance of leaf-shredding invertebrates relates to decomposition processes, and to explore the influence of land use on the in situ feeding rate of *Gammarus pulex*, an important stream detritivore. Leaf litter processing was higher in arable ponds than pastoral ponds but there was no significant difference in leaf processing between coarse and fine mesh bags, suggesting that feeding by invertebrate shredders played a limited role in leaf decomposition in ponds. In contrast, leaf litter processing in streams did not differ between land uses, but it was significantly higher in coarse mesh bags than in fine mesh bags, highlighting the importance of shredding invertebrates in leaf breakdown in streams. There was a positive correlation between the abundance of shredders and leaf mass loss in coarse mesh bags and between the abundance of chironomids and leaf mass loss in fine mesh bags. *Gammarus* feeding rate was lower in arable than pastoral streams and was negatively correlated with ammonia concentration and positively correlated with nitrate concentration.

Most research on the effects of agricultural land use on leaf decomposition has been conducted in streams. To the best of my knowledge, this is the first study investigating impacts of agriculture on leaf decomposition in ponds, and hence direct comparison of the pond results with previous research was limited. One study that has investigated litter decomposition in two types of wetland ponds (uplifted and outwash) on the Copper River Delta in the USA reported similar general findings to those of the current study, that is, decomposition was slow in ponds and invertebrate activity was less important in leaf litter breakdown than microbial breakdown (Tiegs *et al.*, 2013). Several stream studies have highlighted the adverse effect of agricultural land use on leaf decomposition rates (Piscart *et al.*, 2009; Piscart *et al.*, 2011; Jinggut *et al.*, 2012). The results of the current study are similar to those of Hagen *et al.* (2006) and Magbanua *et al.* (2010), who found no significant effect of agricultural land use categories on leaf decomposition rates in streams. However, there was a significant effect of shredder feeding on leaf breakdown; leaf breakdown increasing with increasing shredder abundance. This confirmed findings of other studies (Piscart *et al.*, 2009; Piscart *et al.*, 2011; Rasmussen *et al.*, 2012)

Nutrient enrichment in agricultural catchments can accelerate leaf litter breakdown (Paul *et al.*, 2006), mainly through stimulating aquatic hyphomycetes and accelerating microbial processing (Webster & Benfield, 1986). Higher concentrations of nitrate in arable ponds, measured in 2013 (Chapter 2) and in 2014 (Table 4.4), may partly explain the faster leaf processing in arable compared to pastoral ponds. No significant differences in stream water chemistry were observed in 2013 or 2014, and therefore, no difference in leaf decomposition was expected or observed between arable and pastoral streams. However, across all streams sites a significant positive correlation was observed between nitrate concentration and *Gammarus* feeding rate. *Gammarus* feed on fungally-modified leaf materials and, although all animals were supplied with the same food quality at the start of the deployment, it is possible that food quality varied during the deployment period due to differences in water chemistry between sites. The nutrient enrichment of stream water has been shown to affect microbial activity, particularly high sporulation of aquatic hyphomycetes (Ferreira *et al.*, 2006) and increased fungal biomass (Gulis & Suberkropp, 2003). Fungal biomass is related to food quality; it has been demonstrated that fungi seemed to be more important for *G. pulex* as modifiers of leaf material (Graça *et al.*, 1993) and hence affecting feeding rate.

Mean shredder abundance (in both benthic samples and coarse bags) was similar between arable and pastoral ponds; however, the diversity of shredders was significantly higher in arable ponds. The lack of a significant difference between coarse and fine mesh leaf loss is probably because of the low abundance of shredders in coarse mesh bags, especially in pastoral ponds. In a French pond, Bottollier-Curtet *et al.* (2011) observed low shredder abundance associated with low oxygen and high ammonia concentrations. This result is consistent with the findings of current study. In streams, shredder abundances in both benthic communities and coarse bags were significantly higher in pastoral sites, but no differences were found in either shredder richness or diversity between land uses. The significant decrease in the total abundance of shredders in streams suggested arable land use had some effect, but not enough to reduce leaf processing in arable sites. It has been observed that microbial decomposition was dominant in the leaf breakdown process in highly eutrophic and hypertrophic streams (Lecerf *et al.*, 2006; Baldy *et al.*, 2007).

There were also positive relationships between Chironomidae abundance in coarse mesh bags and total leaf mass loss in coarse mesh bags in pastoral ponds and in arable streams. Further, a significant positive relationship was detected between Chironomidae abundance in fine bags and microbial leaf processing in fine mesh bags in arable ponds. Although most Chironomidae species are mainly considered as collector-gatherers, they can feed on leaf materials (Berg, 1995). The role of Chironomidae in leaf decomposition has been reported (Callisto *et al.*, 2007; da Silveira *et al.*, 2013) and their contribution to leaf processing depends on their density, leaf quality and the presence of other invertebrate consumers that feed on coarse particulate organic matter (Callisto *et al.*, 2007). The results of the current study indicate that total leaf mass loss in coarse bags may be driven by Chironomidae in pastoral ponds and by Chironomidae in a combination with shredding invertebrates in arable streams.

Limnephilidae were the only shredder family that was positively correlated with shredder-mediated mass loss in both ponds and streams. Limnephilidae are known to be non-selective shredders and feed on the whole leaf including mesophyll and venation (Ward & Woods, 1986). In ponds, Asellidae were the most abundant shredder family but there was no significant correlation between Asellidae abundance and shredder-mediated leaf mass loss. This may be explained by the unexpected presence of Asellidae in fine mesh bags which may have affected the shredder-mediated leaf mass loss. There was a positive correlation between the abundance of the amphipods Crangonyctidae and shredder-mediated leaf mass loss in ponds. Crangonyctidae are commonly found in slow flowing rivers and ponds, and can survive in degraded and organic polluted freshwater habitats where they replace Gammaridae (Holland, 1976; MacNeil *et al.*, 2001; MacNeil & Dick, 2014). Gammaridae accounted for 15.6% of individuals present in coarse mesh bags deployed in streams. However, although previous studies have reported that gammarids are key species for leaf litter decomposition in agricultural impacted streams (Piscart *et al.*, 2009), no statistically significant relationship between gammarid abundance and leaf mass was detected in this study.

In situ feeding rate of *G. pulex* deployed at pastoral sites was higher than arable sites, but no significant difference was found between arable and pastoral deployments. This finding is similar to that found by Crane *et al.* (1995) who observed no significant differences in the feeding rates of *G. pulex* deployed in streams located in an agricultural catchment. In contrast, feeding rate of *G. fossarum* was significantly inhibited in 37% of heavily contaminated sites with metal and pesticides (Coulaud *et al.*, 2011). The results of the current study showed that agricultural land use in streams did not inhibit *G. pulex* feeding rate, suggesting that the impact of agriculture was not strong enough to influence feeding activity. In addition, the feeding rate of *G. pulex* increased significantly with increasing nitrate concentrations, whereas it decreased significantly with increasing ammonia concentrations in stream water. It is possible that there may be an interaction between the 'positive' effects of nitrate on feeding via microbially-induced changes in food quality and a 'negative' effect due to ammonia and possibly pesticides. Nitrate addition to stream water has been shown to lead to an increase in microbial activity and hence to affect feeding rate (Graça *et al.*, 1993; Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006). However, ammonia concentrations have been shown previously to have a negative impact on the feeding activity of the amphipod *Eulimnogammarus toletanus* (Alonso & Camargo, 2004), and insecticide application resulted in reduced feeding rates of *G. pulex* (Maltby & Hills, 2008).

4.5. Conclusion

This study demonstrated, through the use of leaf litter decomposition in freshwater, that agricultural land use (arable/pastoral) can alter leaf decomposition in ponds and streams.

However, comparing different types of agricultural practices did not affect leaf processing in streams but a slight change in shredder abundance was observed. This study also highlighted that shredder invertebrates were the key drivers in leaf breakdown in ponds and streams, and consequences of the decline in shredder abundance may affect decomposition rates and nutrient cycling in aquatic ecosystems. Such changes in decomposition rates may affect the availability of organic matter in water, which could affect organism survival and secondary production (Paul *et al.*, 2006).

Chapter 5: The impacts of agricultural land use on algal communities

5.1. Introduction

The effects of agricultural land use on the structure and functioning of benthic invertebrates were presented in Chapters 3 and 4. This chapter addresses the effects of agricultural land use on algal communities. Benthic algae are primary producers that support aquatic food webs and remove nutrients from water (Allan & Castillo, 2007; Cardinale, 2011). Freshwater benthic algae include: green algae (Chlorophyta), yellow-green algae (Chrysophyta), blue-green algae (Cyanophyta or Cyanobacteria), red algae (Rhodophyta) and diatoms (Bacillariophyta) (Lowe & LaLiberte, 2007). Diatoms are one of the major and most widespread and abundant algal groups found in freshwater (Lowe & LaLiberte, 2007). They are diverse communities, have a short generation time, are easily identified compared to other algae or invertebrates (Stevenson & Pan, 1999) and their ecology is well known (Gudmundsdottir *et al.*, 2013). This chapter is focused on total algal biomass and diatom communities in small aquatic habitats located in agricultural landscapes.

Algal biomass, commonly measured as chlorophyll *a* concentration (Gregor & Maršálek, 2004), is affected by abiotic and biotic factors including light, nutrients, temperature, water flow, competition and grazing (Mosisch *et al.*, 2001; Lavoie *et al.*, 2004; Schiller *et al.*, 2007; Urrea-Clos *et al.*, 2014). For example, chlorophyll *a* concentrations in streams in agricultural watersheds in Spain were positively correlated with nitrate concentration, calcium concentration, conductivity and alkalinity (Urrea-Clos *et al.*, 2014).

Experimental studies suggest that the most important nutrients regulating trophic status in streams and rivers are phosphorus (P) and nitrogen (N) (Dodds, 2006). However, the influence of N and P concentrations on algal biomass differs across studies. A meta-analysis of 237 nutrient enrichment studies in temperate streams concluded that algal biomass was N-limited in

16.5% of studies, P-limited in 18.1 % of studies, and limited by both N and P in 23.2 % of studies. In almost half of the studies (i.e. 43 %) there was no evidence that N and P either simulated or inhibited algal growth and in 5 % of studies algal biomass was inhibited by adding N or P (Francoeur, 2001). He demonstrated that it is unlikely for multispecies algal communities to be limited by one nutrient.

The relative importance of nutrients compared to other abiotic factors varies between studies. For example, whereas a study of a subtropical stream concluded that nitrogen concentration was more important than shading in limiting algal production (Mosisch *et al.*, 1999), an in situ experiment in Mediterranean streams in Spain, using nutrient diffusing substrata, concluded that light was the major factor affecting chlorophyll *a* accrual rates and that there was no significant effect of nutrient treatments on algal accrual rates (Schiller *et al.*, 2007). In addition, temperature can affect algal biomass through influencing algal growth rates, which increase with increasing temperature, and it can also affect algal composition through increasing the abundances of cyanobacteria and filamentous green algae in warmer rivers (Allan & Castillo, 2007). Further, the flow of water produces continuous gases and nutrients and therefore enhances the growth of algae by increasing nutrient uptake; however, high flow can decrease algal biomass by disturbing and scouring substrates (Allan & Castillo, 2007).

Biotic factors are also important in controlling algal biomass; grazing invertebrates can reduce algal biomass and make changes in community composition (Rosemond *et al.*, 2000; Hillebrand *et al.*, 2004); they can also influence the nutrient content of the periphyton (Hillebrand *et al.*, 2004). In a meta-analysis on 85 experiments investigating the effects of grazer presence and nutrient supply on algal biomass, Hillebrand (2002) concluded that both nutrient and grazers had strong effect on algal biomass and that grazers had greater effects than nutrients.

Diatoms respond rapidly to environmental changes and are sensitive to many environmental variables (e.g. pH, nutrients, pollutants), and they are therefore used in biomonitoring (Kelly &

Whitton, 1995; Stevenson & Pan, 1999; Kelly *et al.*, 2008). It has been argued that because diatom community composition is more strongly correlated with water chemistry than that of other algal groups, they are ideal indicators of trophic status in streams and rivers (Kelly & Whitton, 1995; Stevenson & Pan, 1999; Kelly *et al.*, 2008). An example is the trophic diatom index (TDI) which is a monitoring index used to indicate the trophic status in rivers based on diatom community composition and their species tolerance to nutrient concentrations (Kelly & Whitton, 1995). The TDI was calibrated initially to phosphorous concentrations, but then it has been calibrated to both nitrogen and phosphorus (Kelly *et al.*, 2008). Low index scores indicate low tropic status and high scores indicate high trophic status and eutrophication (Kelly *et al.*, 2008). Recently, the TDI method has been developed for lakes (Bennion *et al.*, 2014) based on TDI for rivers and streams.

A large body of research has reported the effect of nutrient enrichment on diatom species composition (Jüttner *et al.*, 2003; Bellinger *et al.*, 2006; Della Bella & Mancini, 2009; Gudmundsdottir *et al.*, 2013). In Iceland, Gudmundsdottir *et al.* (2013) studied the effects of nitrogen enrichment on diatom communities in sub-arctic streams by adding ammonium nitrate to pristine streams. They found that nitrogen enrichment significantly increased the diatom densities and decreased the diversity of diatoms, but the trophic diatom index (TDI) was not affected by nitrogen addition. In African tropical streams, Bellinger *et al.* (2006) found that the deforested streams, which had significantly high nutrient concentrations, had significantly more diatoms and non-diatom genera; however, diatom species richness and diversity did not significantly differ between forested and deforested streams and the TDI was significantly higher in deforested streams. A study of 21 permanent ponds in Italy by Della Bella and Mancini (2009) analysing diatoms in reference ponds and agricultural or human impacted ponds found that degraded ponds had the highest nitrate concentrations and that benthic diatom species were significantly higher in intermediate impacted ponds than reference and degraded ponds. In contrast, Lavoie *et al.* (2004) analysed the diatom assemblage in Canadian streams and found that conductivity, pH and suspended solids were the main environmental factors explaining differences in diatom assemblages among sites and that variation in nitrogen and phosphorus concentrations did not account for differences in diatom community structure.

Nutrient enrichment of freshwater alters benthic algae, shifting communities from diatom dominated communities to communities dominated by filamentous green algae such as *Cladophora* sp. (Dodds, 2006; Cardinale, 2011; Gudmundsdottir *et al.*, 2013). A large number of studies investigated the effects of increasing nutrients resulting from agriculture on benthic algal communities, including diatoms (Lavoie *et al.*, 2004; Della Bella & Mancini, 2009; Urrea & Sabater, 2009; Yu & Lin, 2009; Gabel *et al.*, 2012; Johnson & Angeler, 2014; Urrea-Clos *et al.*, 2014). For example, Johnson and Angeler (2014) investigated the effects of agricultural land use on stream communities using European datasets of stream fish, invertebrates, macrophytes and benthic diatoms. The main effect of agriculture on water chemistry was an increase in total phosphorus, which was associated with a significant increase in diatom species richness, diatom diversity and evenness. In subtropical streams in Taiwan, Yu and Lin (2009) found that the effects of agriculture on epilithic algal communities were significant and varied with the area of agriculture in the catchment and that algal biomass was significantly higher in a stream with larger area of agriculture and higher concentrations of nitrate and nitrite. In an agricultural landscape consisting of dairy farms and row crops in USA, Gabel *et al.* (2012) investigated the effectiveness of agricultural best management practices (including riparian plantation, stream bank fencing, improved manure storage and barnyard improvements) on stream diatoms. They found that benthic algal biomass and trophic diatom index were significantly higher and diatom species richness was lower in streams lacking management practices.

Pesticide use is another major impact of agriculture on freshwater communities. A decrease in algal biomass, measured as chlorophyll *a*, was observed when algae were exposed to the herbicide (atrazine) at different concentrations 10–1000 μg/l (Berard, 1996; Carder & Hoagland, 1998). In a microcosm study, Schmitt-Jansen and Altenburger (2005) reported an increase in green algae at low concentrations of the herbicide (isoproturon) and there was no inhibition in algal biomass. They concluded that herbicide concentrations in rivers may change the structure of periphyton communities even when the concentrations do not exceed acute toxicity levels (Schmitt-Jansen & Altenburger, 2005). Debenest *et al.* (2010) suggested that green algae could be more tolerant to isoproturon than diatoms. Furthermore, herbicide exposure can affect the diversity of diatom communities (Berard *et al.*, 2004; Schmitt-Jansen & Altenburger, 2005) and increase the number of abnormal frustules in the assemblage (Debenest *et al.*, 2008). In a microcosm study, the exposure of isoproturon resulted in sensitive species replaced by tolerant species and an increase in small species such as *Achnanthes minutissima*, *Navicula minima*, and *Navicula halophile* (Schmitt-Jansen & Altenburger, 2005).

Generally, a large body of research investigated the effects of agricultural land use on freshwater benthic algae in comparison with other land use patterns such as forested or urban, but they did not take into account different types of agriculture land use. The aim of this chapter is to quantify the relative importance of different types of agricultural land use on algal communities in freshwater habitats. This was achieved by sampling algal communities in ponds and streams across arable-pastoral catchments. The objectives of this study were to: 1) compare algal biomass (measured as chlorophyll *a* concentration) between arable and pastoral sites in ponds and streams, and 2) to compare diatom communities (species richness, diversity, evenness and composition) and trophic diatom index between arable and pastoral water bodies.

5.2. Methods

5.2.1. Study sites

Twelve ponds (6 arable and 6 pastoral) and twelve streams (6 arable and 6 pastoral) were sampled in Eye Brook and Stonton Brook catchments in Leicestershire (see Chapter 2 for detailed site descriptions). Water chemical data were taken by the Water Friendly farming (WFF) project in 2013, which was the same year as the collection of algae for the current study (see Chapter 2, Section 2.3 and Table 2.4 for detailed results). Estimated shade (%) is presented in Table 2.1 (Chapter 2).

5.2.2. Sampling algal communities

Two hundred and sixteen unglazed tiles (45 mm × 45 mm) were deployed in April 2013 to provide artificial substrates for algal colonisation (Lowe & Pan, 1996; Kelly *et al.*, 2001). Nine tiles were deployed per site: five tiles for chlorophyll *a* analysis and four tiles for diatom identification. Each tile was attached to a piece of plastic mesh (pore size 7 mm × 5 mm) using fishing line. In ponds, mesh pieces were placed on top of a brick, but in streams, they were placed immediately on the stream bed and secured using tent pegs. Each brick or mesh piece was tied to a wooden stake or a tree using fishing line. After six weeks, all tiles were collected and the upper surface of each tile was brushed with a toothbrush to remove the algal film, which was put into a labelled sample bottle containing 30 ml of site water. All sample bottles were transferred to the laboratory in a cool box and kept in the fridge at 5°C until processing the next day.

5.2.3. Chlorophyll *a* analysis

Chlorophyll *a* concentration, which is a measure of total algal biomass, was analysed using a combined method from Gregor and Maršálek (2004) and Horne (2009). The contents of each sample bottle were filtered through Whatman Number 1 filter paper and all filter papers were stored in a freezer at (−18 °C) until the analysis the following day. After thawing, each filter paper was placed in a foil covered test tube containing 10 ml of 90% ethanol. All tubes were placed in a water bath at 80°C for 10 minutes. After cooling for 30 minutes, 3 ml of the sample was placed in a cuvette and read at 664 nm and 750 nm using a Perkin Elmer Lambda 40 UV-VIS Spectrophotometer (90% ethanol was used as a blank sample). Chlorophyll *a* concentration was calculated using the following equation:

$$Chlorophyll\ a\ (\mu g/cm^2) = E \times \frac{A_{664nm} - A_{750nm}}{Area} \times V_{extract} \times DF \times L \qquad \text{Equation 5.1}$$

where E is the extinction coefficient for chlorophyll a in 90% ethanol at 664nm (i.e. 12.8), A_{664nm} and A_{750nm} are absorption readings at 664 nm and 750 nm respectively, $V_{extract}$ is the volume of extract (i.e. 10 ml), DF is the dilution factor (i.e. 1), $Area$ is the sampled area of rock/tile (i.e. 20.25 cm^2) and L is the cuvette path length (i.e. 1 cm).

5.2.4. Diatom preparation and identification

The hot peroxide method (Kelly *et al.*, 2001) was used to prepare permanent microscopic slides for diatom identification and counting. Fifteen millilitres of hydrogen peroxide was added to a test tube containing 10 ml of diatom sample in a fume cupboard. All tubes were heated in a water bath at 80°C for 5-6 hours. After cooling, a few drops of hydrochloric acid were added to remove any remaining hydrogen peroxide and carbonates. Each solution was transferred to a centrifuge tube (15 ml Falcon tube), topped up with water to 14 ml, and centrifuged at a speed of 3000-3200 rpm for 5 minutes (Benchtop Centrifuge, Centaur 2 MS, PAT 01341). After centrifuging, the supernatant was decanted off leaving behind the algal pellet, more water was added and the centrifugation process was repeated twice more. To prepare a slide, excess water was removed from a centrifuge tube, and the remaining solution (1-2 ml) was shaken to break up the algal pellet. A drop of the diatom suspension was placed on a clean coverslip and heated on a hotplate at 60°C. Once the drop had dried, the coverslip was mounted on a clean microscopic slide using Naphrax. Diatoms were examined under a microscope at 1000 ×

magnification, and up to 400 valves were counted and identified to species level using appropriate keys (Kelly, 2000; Kelly *et al.*, 2005).

5.2.5. Data analysis

Chlorophyll a concentration and diatom species richness, Shannon diversity index and species evenness were compared between arable and pastoral sites using two-sample *t*-tests to determine whether there were significant differences in Chlorophyll a or diatom communities between arable and pastoral land use in ponds and streams. Chlorophyll a concentration in ponds was natural logarithm transformed before analysis to achieve normality.

The trophic diatom index (TDI) is a monitoring index used to indicate the trophic status in rivers and based on diatom community composition and their species tolerance to nutrient concentrations (Kelly & Whitton, 1995). It was revised by Kelly *et al.* (2008) and calculated using Equations 5.2 and 5.3:

$$WMS = \frac{\sum_{j=1}^{n} a_j s_j}{\sum_{j=1}^{n} a_j} \qquad \text{Equation 5.2}$$

where *WMS* is weighted mean score, a_j is the abundance of species j in the sample and s_j is the nutrient sensitivity (1-5) of species j.

$$TDI = (WMS \times 25) - 25 \qquad \text{Equation 5.3}$$

TDI has a value between 0 (very low nutrient) indicating low trophic status and 100 (very high nutrient) indicating high trophic status (Kelly *et al.*, 2008). Two-sample *t*-test was used for significant differences in TDI between arable and pastoral sites.

Simple linear regressions were used to explore the effect of water nutrients (mainly total nitrogen TN and total phosphorus TP) on chlorophyll a concentration and TDI. Chlorophyll a, TN and TP were natural logarithm transformed before analyses.

Principal component analysis (PCA) on covariance matrix was used to investigate responses of diatom communities in ponds and streams to arable and pastoral land use. Sites were compared by pooling diatom samples within each site. Diatom species were excluded from the analysis if they constituted less than 1% of the overall abundance in order to reduce the influence of rare species. Analysis of Similarity (ANOSIM) (Clarke, 1993) was used to examine significant differences in the composition of diatom communities in ponds and streams between arable and pastoral sites. Similarity Percentage Analysis (SIMPER) (Clarke, 1993) was used to determine which diatom species were responsible for differences between communities in different land uses and to calculate the percentage contribution of each diatom species to the overall dissimilarity (Bray-Curtis). Abundances of diatom species were ln (x+1) transformed prior to analysis. Statistical analyses were carried out using R (R Core Team, 2013). In R, the package ggplot2 (Wickham, 2009) was used for producing graphics, and the package vegan (Oksanen *et al.*, 2013) was used for running ANOISM and SIMPER analyses.

5.3. Results

5.3.1. Chlorophyll *a* concentration

The chlorophyll *a* concentrations of algal communities sampled from ponds ranged from 0.81 to 2.16 µg/cm^2 for arable sites, and from 0.20 to 0.39 µg/cm^2 for pastoral sites. In streams, arable sites had chlorophyll *a* concentrations ranging from 0.52 to 3.35 µg/cm^2 compared to 0.37 to 2.00 µg/cm^2 for pastoral sites. For both ponds and streams, chlorophyll *a* concentrations were higher in arable sites than pastoral sites, but these differences were only statistically significant for ponds (two-sample *t*-test: $t_{10} = 9.09$, $p < 0.001$; Figure 5.1). In addition, there was no relationship between chlorophyll *a* concentrations and shade percentage in either ponds or streams (all: $F_{1,10} < 1.34$, $p > 0.05$).

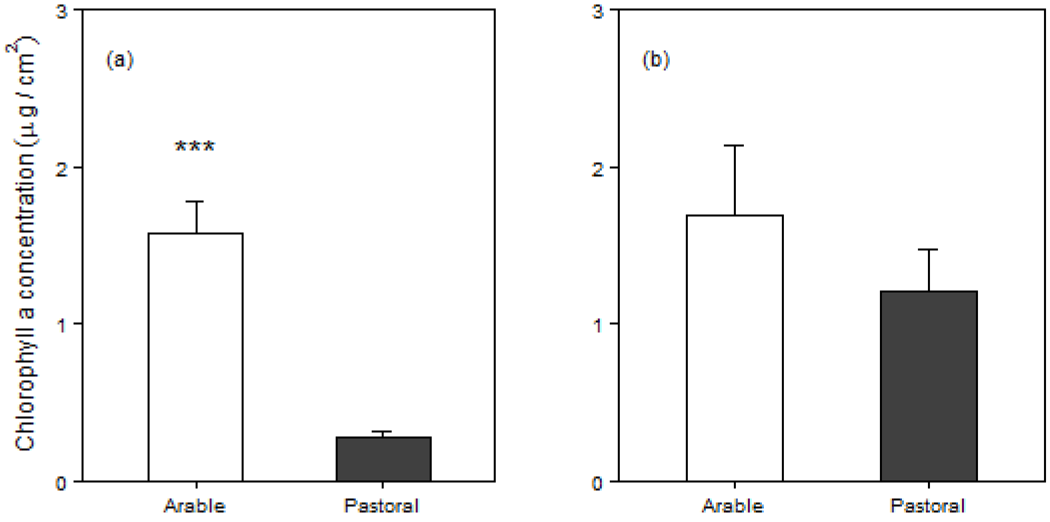

Figure 5.1 Mean (+ SE) chlorophyll *a* concentration in arable (white bars) and pastoral (black bars) in (a) ponds and (b) streams. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

5.3.2. Diatom species richness, diversity, evenness and TDI

A total of 91 diatom species were identified in 72 samples collected from ponds and streams (Appendix B, Table B1 and Table B2, respectively). Ponds had more species than streams; there were 87 species in ponds compared to only 45 species in streams. For both ponds and streams, most diatom species occurred in both arable and pastoral sites (Table 5.1 and 5.2). Bray-Curtis similarity index was 0.71 for pond diatoms and 0.75 for stream diatoms.

Arable ponds had lower diatom species richness, species diversity and species evenness than pastoral ponds. These differences between land uses were marginally significant for species richness (two-sample *t*-test: $t_{10} = 1.88$, $p = 0.09$; Figure 5.2a) and significant for both diversity index (two-sample *t*-test: $t_{10} = 3.33$, $p < 0.01$; Figure 5.2c) and species evenness (two-sample *t*-test: $t_{10} = 3.39$, $p < 0.01$; Figure 5.2e). In streams, there were no significant differences between arable and pastoral sites in either species richness or Shannon diversity index or species evenness (two-sample *t*-test: all $t_{10} < 1.78$, $p > 0.05$; Figure 5.2).

The trophic diatom index (TDI) in ponds ranged from 26.6 to 42.2 for arable sites, and from 39.8 to 57.2 for pastoral sites. TDI was significantly higher in pastoral ponds than arable ponds (two-sample *t*-test: $t_{10} = 5.37$, $p < 0.001$; Figure 5.3a). In streams, TDI values ranged from 37.3 to 65.3 for arable, and from 29.1 to 51.8 for pastoral streams; however, there was no statistically significant difference between arable and pastoral sites (two-sample *t*-test: $t_{10} = 1.43$, $p > 0.05$; Figure 5.3b).

Table 5.1 Diatom species identified in arable and pastoral ponds.

No.	Arable and pastoral ponds	Arable ponds only	Pastoral ponds only
1	*Achnanthidium minutissimum*	*Cyclotella meneghiniana*	*Achnanthes oblongella*
2	*Amphora libyca*	*Cyclotella sp.*	*Caloneis bacillum*
3	*Amphora pediculus*	*Cymatopleura solea*	*Craticula ambigua*
4	*Cocconeis placentula*	*Cymbella microcephala*	*Cymbella cuspidata*
5	*Cymbella cistula*	*Diatoma mesodon*	*Diadesmis contenta*
6	*Cymbella lanceolata*	*Diatoma tenue*	*Diploneis elliptica*
7	*Diploneis oblongella*	*Eunotia minor*	*Gomphonema gracile*
8	*Encyonema silesiacum*	*Melosira varians*	*Gomphonema olivaceum*
9	*Eunotia bilunaris*	*Navicula capitata*	*Lemnicola hungarica*
10	*Eunotia exigua*	*Navicula menisculus*	*Navicula cari*
11	*Fragilaria capucina*	*Nitzschia acicularis*	*Navicula molestiformis*
12	*Fragilaria tenera*	*Nitzschia sigma*	*Navicula slesvicensis*
13	*Fragilaria vaucheriae*	*Placoneis clementis*	*Navicula trivialis*
14	*Frustulia vulgaris*	*Reimeria sinuata*	*Navicula veneta*
15	*Gomphonema acuminatum*	*Sellaphora seminulum*	*Nitzschia amphibia*
16	*Gomphonema angustatum*	*Synedra parasitica*	*Nitzschia capitellata*
17	*Gomphonema clavatum*		*Nitzschia sp.*
18	*Gomphonema parvulum*		*Placoneis elginensis*
19	*Gomphonema truncatum*		*Planothidium delicatulum*
20	*Gyrosigma acuminatum*		*Sellaphora pupula*
21	*Hantzschia amphioxys*		*Stauroneis smithii*
22	*Luticola ventricosa*		*Surirella minuta*
23	*Meridion circulare*		*Tryblionella hungarica*
24	*Navicula atomus*		
25	*Navicula capitatoradiata*		
26	*Navicula cryptocephala*		
27	*Navicula cryptotenella*		
28	*Navicula gregaria*		
29	*Navicula lanceolata*		
30	*Navicula minima*		
31	*Navicula radiosa*		
32	*Navicula tripunctata*		
33	*Nitzschia dissipata*		
34	*Nitzschia linearis*		
35	*Nitzschia palea*		
36	*Nitzschia pusilla*		
37	*Pinnularia appendiculata*		
38	*Pinnularia subcapitata*		
39	*Pinnularia viridis*		
40	*Planothidium frequentissimum*		
41	*Planothidium lanceolata*		
42	*Stauroneis anceps*		
43	*Stauroneis kriegeri*		
44	*Stauroneis phoenicenteron*		
45	*Surirella angusta*		
46	*Surirella brebisonii*		
47	*Synedra acus*		
48	*Tryblionella apiculata*		

Table 5.2 Diatom species identified in arable and pastoral streams.

No.	Arable and pastoral streams	Arable streams only	Pastoral stream only
1	*Achnanthidium minutissimum*	*Caloneis bacillum*	*Amphora libyca*
2	*Amphora pediculus*	*Diatoma mesodon*	*Diploneis elliptica*
3	*Cocconeis placentula*	*Encyonema minutum*	*Fragilaria capucina*
4	*Diploneis oblongella*	*Encyonema silesiacum*	*Fragilaria vaucheriae*
5	*Frustulia vulgaris*	*Navicula atomus*	*Gomphonema gracile*
6	*Gomphonema angustatum*	*Navicula capitata*	*Navicula cryptocephala*
7	*Gomphonema olivaceum*	*Pseudostaurosira brevistriata*	*Sellaphora pupula*
8	*Gomphonema parvulum*	*Stauroneis anceps*	*Stauroneis smithii*
9	*Hantzschia amphioxys*	*Tryblionella hungarica*	*Synedra ulna*
10	*Meridion circulare*		
11	*Navicula cryptotenella*		
12	*Navicula gregaria*		
13	*Navicula lanceolata*		
14	*Navicula minima*		
15	*Navicula menisculus*		
16	*Navicula molestiformis*		
17	*Navicula tripunctata*		
18	*Nitzschia amphibia*		
19	*Nitzschia dissipata*		
20	*Nitzschia linearis*		
21	*Nitzschia palea*		
22	*Planothidium frequentissimum*		
23	*Planothidium lanceolata*		
24	*Reimeria sinuata*		
25	*Rhoicosphenia abbreviata*		
26	*Surirella angusta*		
27	*Surirella brebisonii*		

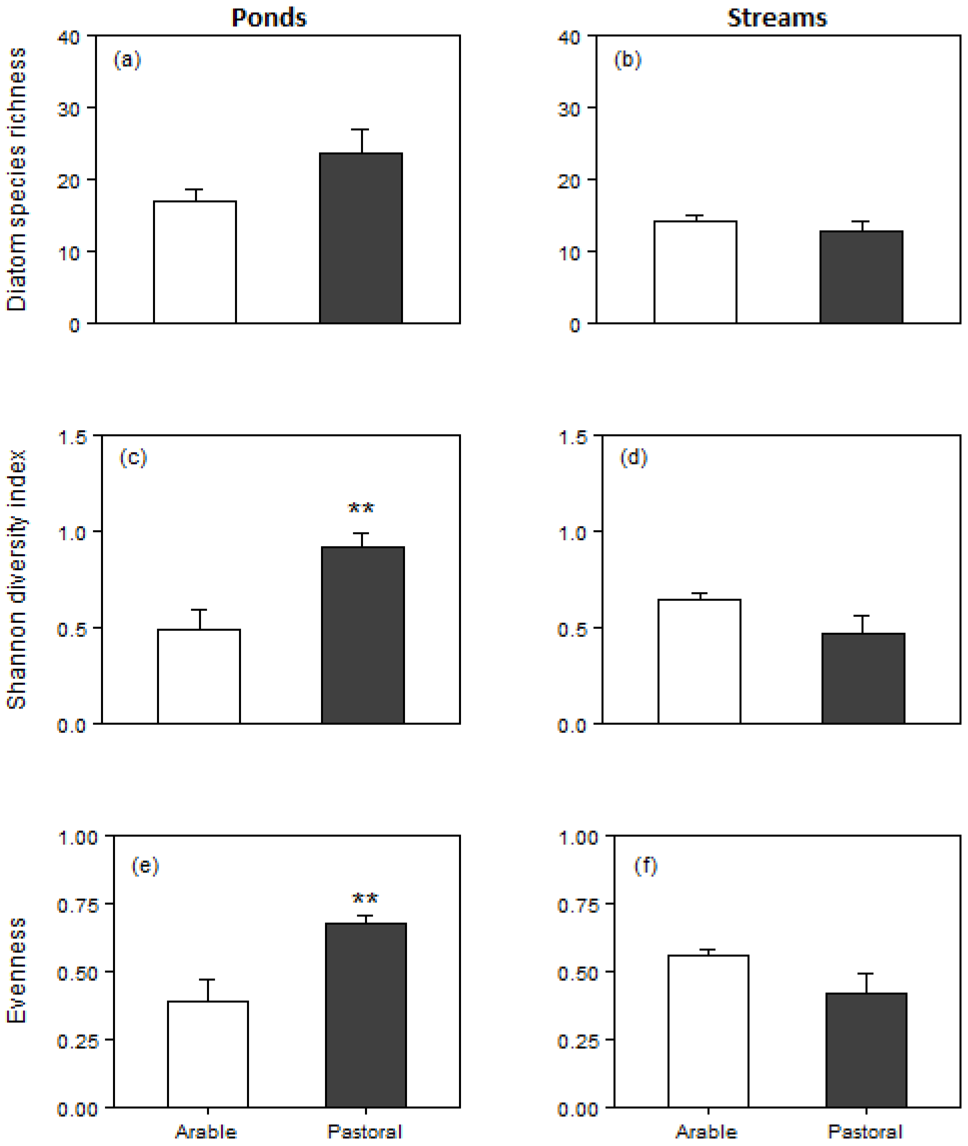

Figure 5.2 Mean (+ SE) diatom species richness (a, b), Shannon diversity index (c, d) and evenness (e, f) in arable (white bars) and pastoral (black bars) in ponds on left side and streams on right side. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

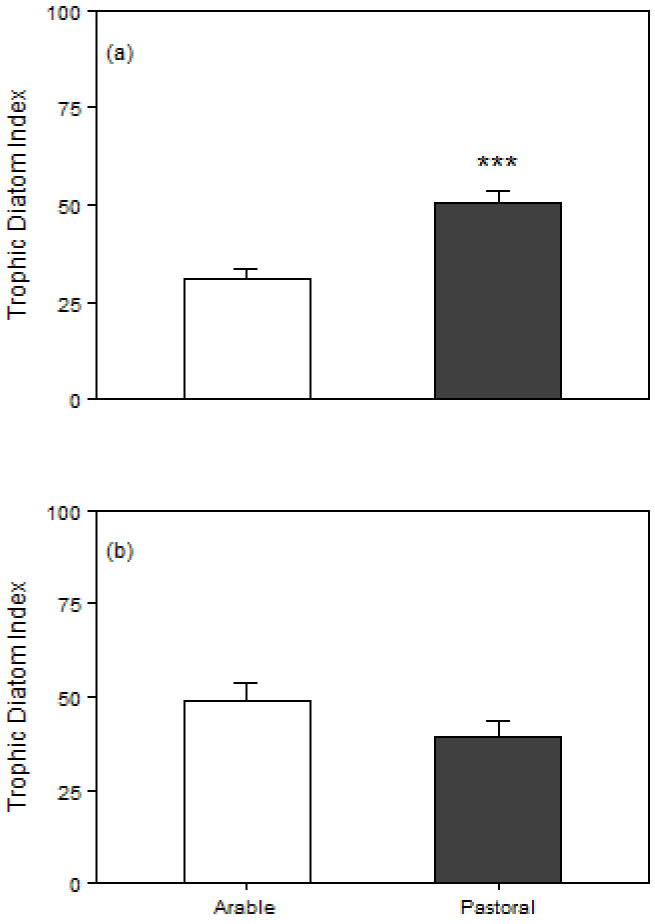

Figure 5.3 Mean (+ SE) trophic diatom index in arable (white bars) and pastoral (black bars) in (a) ponds and (b) streams. Asterisks indicate significant differences between arable and pastoral sites (two-sample *t*-test).

5.3.3. The relationship between nutrients and chlorophyll *a* and TDI

In ponds, there was a significant positive relationship between chlorophyll *a* concentrations and total nitrogen ($F_{1,\ 10} = 8.61$, $p < 0.05$, $r^2 = 0.46$; Figure 5.4a) and a significant negative relationship with total phosphorus ($F_{1,\ 10} = 22.2$, $p < 0.001$, $r^2 = 0.69$; Figure 5.4b). However, when compared within land use type, these relationships were only significant for chlorophyll *a* and total nitrogen in arable ponds ($F_{1,\ 4} = 8.84$, $p < 0.05$, $r^2 = 0.69$). There was a marginally non-significant negative relationship between trophic diatom index and total nitrogen ($F_{1,\ 10} = 4.4$, $p = 0.06$, $r^2 = 0.31$; Figure 5.4c), and a significant positive relationship with total phosphorus ($F_{1,\ 10} = 32.9$, $p < 0.001$, $r^2 = 0.77$; Figure 5.4d). Comparing within land use type, however, these relationships were only significant for trophic diatom index and total phosphorus in arable ponds ($F_{1,\ 4} = 9.87$, $p < 0.05$, $r^2 = 0.71$).

In streams, no significant relationships were found between chlorophyll *a* concentrations and total nitrogen or total phosphorus (all: $F_{1,\ 10} < 2.26$, $p > 0.05$; Figure 5.5a and 5.5b). In addition, no significant relationships were detected when data were analysed for each land use separately. There was no significant relationship between trophic diatom index and total nitrogen ($F_{1,\ 10} = 0.66$, $p > 0.05$, $r^2 = 0.07$; Figure 5.5c), but there was a marginally non-significant positive relationship between total phosphorus and trophic diatom index ($F_{1,\ 10} = 4.42$, $p = 0.06$, $r^2 = 0.33$; Figure 5.5d). Comparing within land use type, however, these relationships were only marginally non-significant for trophic diatom index and total nitrogen in arable streams ($F_{1,\ 4} = 5.37$, $p = 0.08$, $r^2 = 0.57$).

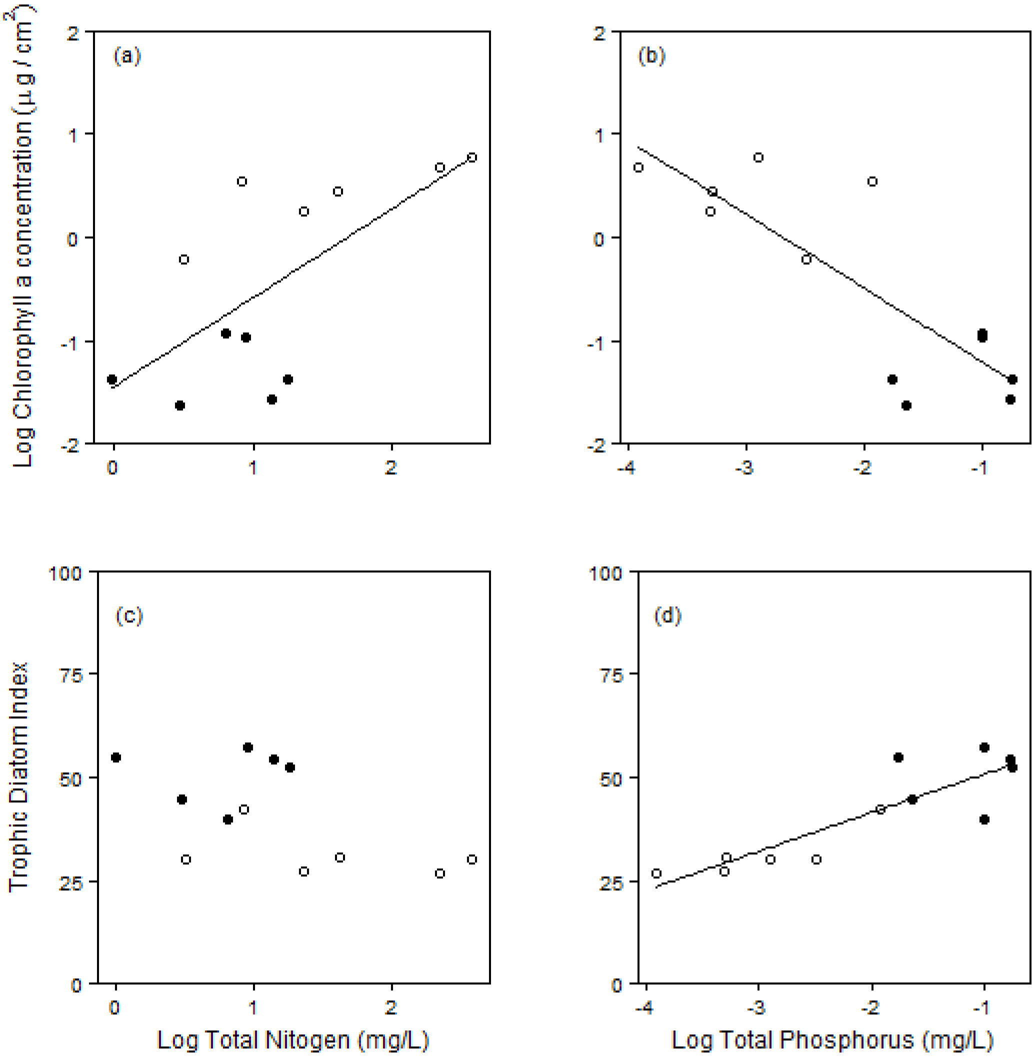

Figure 5.4 Relationships between chlorophyll *a* concentration in ponds and (a) total nitrogen and (b) total phosphorus; and relationships between trophic diatom index in ponds and (c) total nitrogen and (d) total phosphorus. Open circles indicate arable ponds and closed circles indicate pastoral ponds.

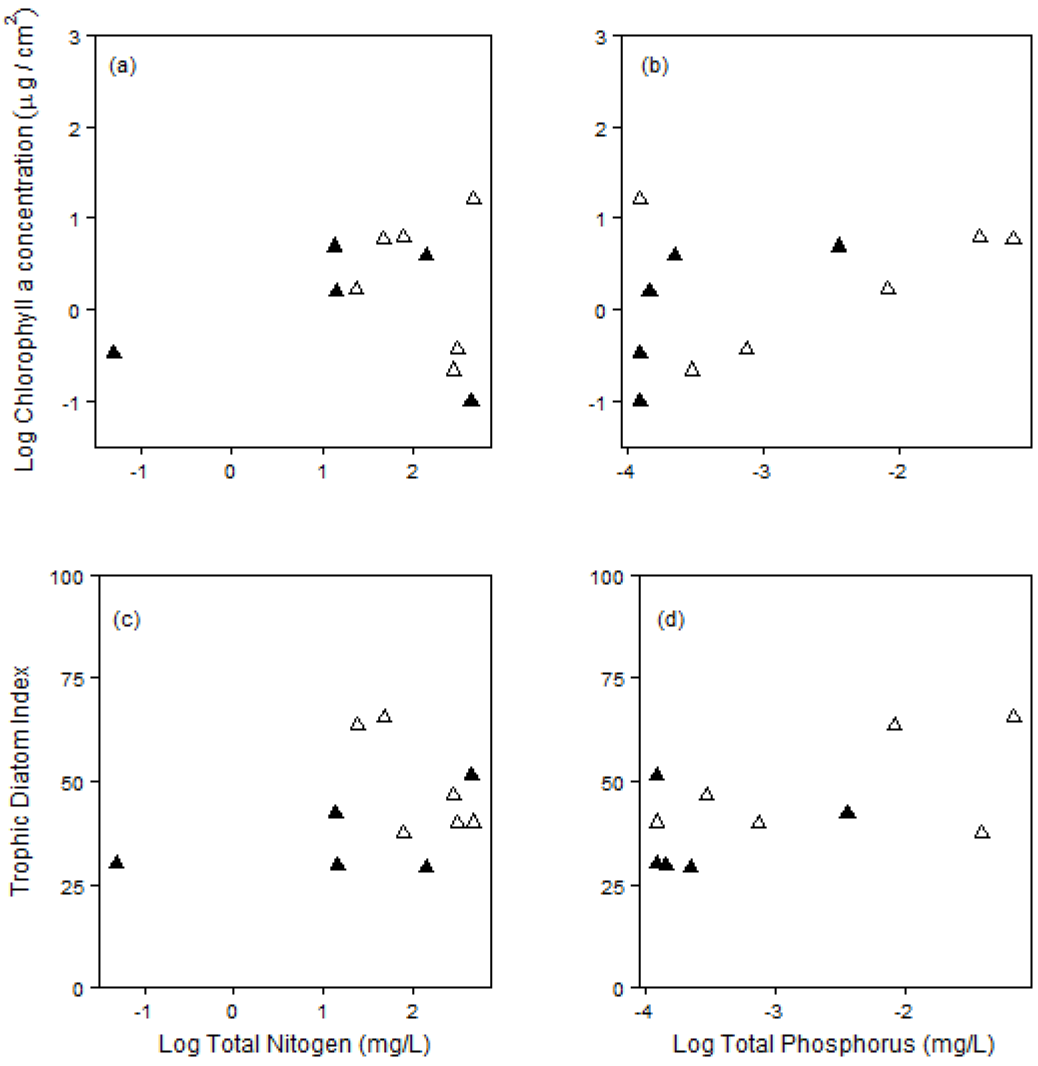

Figure 5.5 Relationships between chlorophyll *a* concentration in streams and (a) total nitrogen and (b) total phosphorus; and relationships between trophic diatom index in streams and (c) total nitrogen and (d) total phosphorus. Open triangles indicate arable streams and closed triangles indicate pastoral streams. The stream PS2 was omitted from the analysis due to no chemical data was available.

5.3.4. Diatom community composition

The results of the principal component analysis (PCA) of diatom communities in ponds and streams are shown in Figure 5.6. For the pond diatom community, the first and second components explained 51.14% of the total variation, and arable and pastoral ponds were separated along the first component (Figure 5.6a). The diatom species associated with arable ponds were *Achnanthidium minutissimum*, *Fragilaria capucina*, *Synedra acus*, *Cyclotella sp.* and *Gomphonema truncatum*. Five of the six arable ponds had similar diatom communities whereas the arable pond AP2 had a different community with abundant *Eunotia bilunaris* and *Gomphonema parvulum*. The diatom communities in five of six pastoral ponds were similar to each other and were characterised by a high abundance of *Planothidium frequentissimum*, *Planothidium lanceolate*, *Navicula gregaria*, *Navicula minima*, *Nitzschia palea*, *Gomphonema angustatum* and *Sellaphora pupula*. The community in the pastoral pond PP6 was distinct from the others and associated with *Nitzschia sp.* and *Navicula cryptocephala*.

For stream diatom communitites, the first and second components of PCA explained 30.2% and 20.72% of the total variation, respectively (Figure 5.6b). As can be seen in Figure 5.6b, communities in arable and pastoral streams overlapped with each other. The most abundant diatom species in both arable and pastoral streams were *Achnanthidium minutissimum*, *Navicula lanceolate* and *Planothidium lanceolate*. Arable streams were associated with high abundance of *Nitzschia dissipata*, *Navicula gregaria*, *Cocconeis placentula* and *Surirella brebisonii*, while pastoral streams had a high abundance of *Amphora pediculus*. Although the pond (AP5) and the stream (AS5) were classified as 'mixed' sites (Chapter 2), they did not differ from the rest of other arable sites in their diatom community composition.

Analysis of similarity (ANOSIM) indicated that the diatom communities were significantly different between arable and pastoral ponds (R = 0.5, p = 0.007). The results of Similarity Percentages analysis (SIMPER) identified the species that are responsible for the features distinguishing between arable and pastoral ponds (Table 5.3). The five species accounted for

42% of differences between arable and pastoral ponds were *Planothidium frequentissimum*, *Planothidium lanceolate*, *Navicula gregaria*, *Eunotia bilunaris* and *Gomphonema parvulum*.

The results of ANOSIM for stream diatoms revealed that there was no significant difference in the diatom communities between arable and pastoral streams (R = 0.004, p = 0.4). SIMPER analysis indicated that the top five diatom species in streams that contributed to 49% of dissimilarities between arable and pastoral streams were *Navicula lanceolate*, *Nitzschia dissipata*, *Navicula gregaria*, *Planothidium lanceolate* and *Cocconeis placentula*, all of which were more abundant in arable streams (Table 5.3).

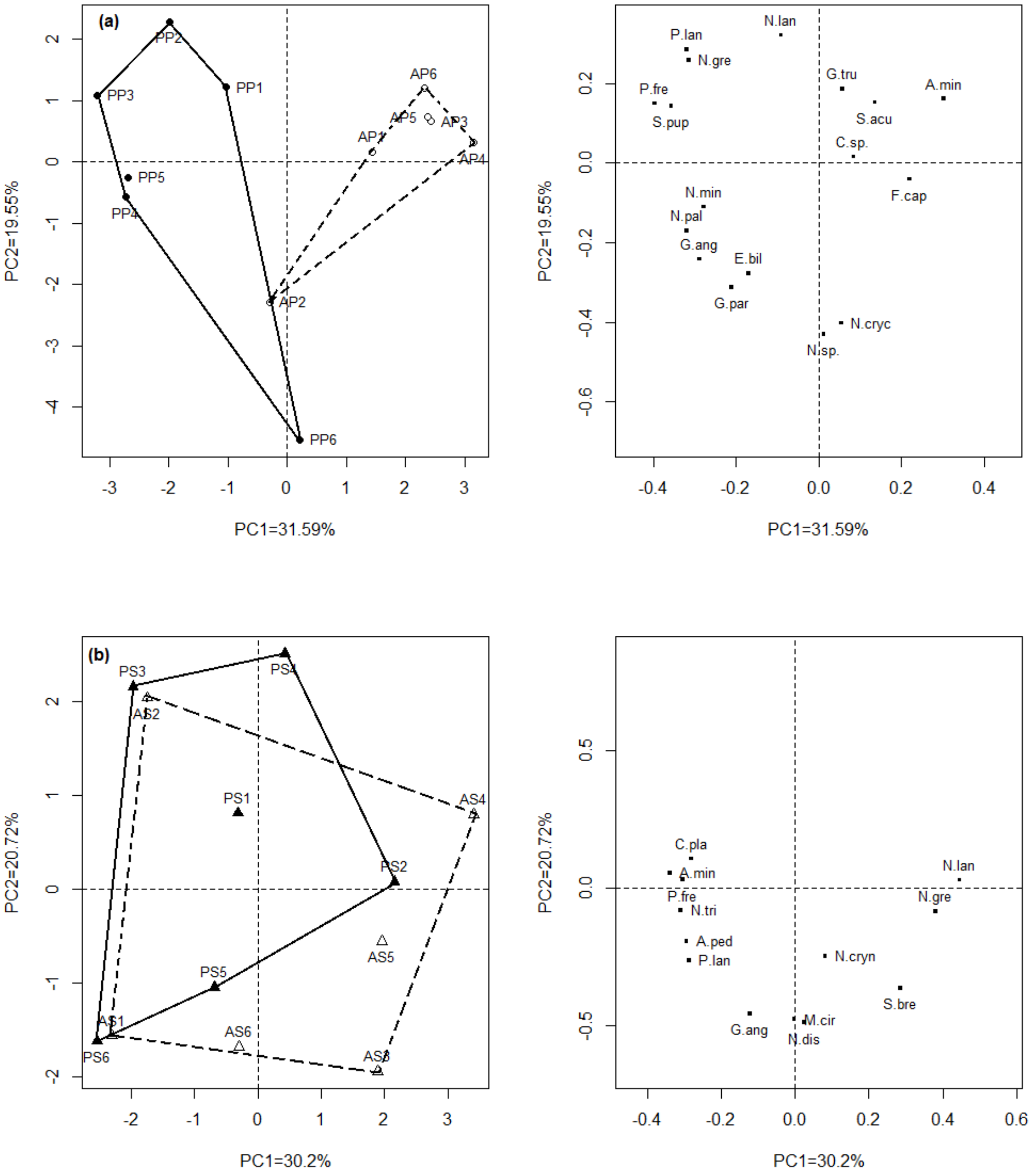

Figure 5.6 Principal component analysis of the diatom communities in (a) ponds and (b) streams in arable (open circles and triangles and dashed lines) and pastoral (closed circles and triangles and solid lines). Species codes are A.min: *Achnanthidium minutissimum*, A.ped: *Amphora pediculus*, C.pla: *Cocconeis placentula*, C.sp.: *Cyclotella sp.*, E.bil: *Eunotia bilunaris*, F.cap: *Fragilaria capucina*, G.ang: *Gomphonema angustatum*, G.par: *Gomphonema parvulum*, G.tru: *Gomphonema truncatum*, M.cir: *Meridion circulare*, N.cryc: *Navicula cryptocephala*, N.cryn: *Navicula cryptonella*, N.gre: *Navicula gregaria*, N.lan: *Navicula lanceolate*, N.min: *Navicula minima*, N.tri: *Navicula tripunctata*, N.dis: *Nitzschia dissipata*, N.pal: *Nitzschia palea*, N.sp.: *Nitzschia sp.*, P.fre: *Planothidium frequentissimum*, P.lan: *Planothidium lanceolate*, S.pup: *Sellaphora pupula*, S.bre: *Surirella brebisonii*, S.acu: *Synedra acus*.

Table 5.3 Similarity of percentages (SIMPER) analysis of diatom communities using Bray-Curtis dissimilarity between arable and pastoral ponds or streams. Contribution % is percent contribution a species makes to the overall dissimilarity between the two communities. Cumulative % is the ordered percentage of cumulative contribution.

Diatom species	**Contribution%**	**Cumulative%**	**Average Log Abundance**	
			Arable	Pastoral
Ponds				
Planothidium frequentissimum	10.97	10.97	0.75	3.51
Planothidium lanceolate	8.97	19.94	0.50	2.66
Navicula gregaria	8.43	28.37	0.92	2.89
Eunotia bilunaris	7.32	35.69	1.43	1.68
Gomphonema parvulum	6.54	42.23	0.90	1.87
Sellaphora pupula	6.39	48.62	0.00	1.63
Gomphonema angustatum	6.18	54.8	1.03	2.21
Fragilaria capucina	6.09	60.89	1.86	1.12
Navicula minima	5.75	66.64	0.59	2.03
Nitzschia palea	5.32	71.96	1.30	2.54
Navicula lanceolate	5.32	77.28	0.87	1.42
Synedra acus	5.22	82.5	1.42	0.48
Achnanthidium minutissimum	4.44	86.94	5.22	4.34
Navicula cryptocephala	3.49	90.43	0.23	0.75
Gomphonema truncatum	3.42	93.85	0.66	0.45
Nitzschia sp.	3.37	97.22	0.00	0.85
Cyclotella sp.	2.78	100	0.78	0.00
Streams				
Navicula lanceolate	12.73	12.73	2.92	2.20
Nitzschia dissipata	10.05	22.78	2.48	0.88
Navicula gregaria	9.86	32.64	2.72	1.31
Planothidium lanceolate	8.36	41	2.73	1.87
Cocconeis placentula	7.89	48.89	1.26	1.11
Meridion circulare	7.74	56.63	2.13	1.92
Surirella brebisonii	7.37	64	2.30	1.57
Achnanthidium minutissimum	6.45	70.45	4.47	5.50
Amphora pediculus	6.31	76.76	1.88	2.26
Navicula cryptocephala	6.28	83.04	1.05	0.49
Planothidium frequentissimum	6.03	89.07	1.47	1.50
Gomphonema angustatum	5.48	94.55	1.40	1.83
Navicula tripunctata	5.45	100	1.45	1.30

5.4. Discussion

The objectives of this study were to compare algal biomass (measured as chlorophyll *a* concentration) between arable and pastoral sites in ponds and streams, and to compare diatom communities (species richness, diversity, evenness and composition) and trophic diatom index between arable and pastoral water bodies. Algal biomass was higher in arable ponds and streams than pastoral ponds and streams, but these differences between land uses were only statistically significant for ponds. Arable ponds had lower diatom species richness, diversity, evenness and trophic diatom index than pastoral ponds, while in streams these differences between land uses were non-significant. Diatom community composition in arable ponds was significantly different from pastoral ponds. In contrast, there were no significant differences in the composition of diatom communities between arable and pastoral streams.

Several studies analysing the effects of agricultural land use on algal biomass reported that chlorophyll *a* concentrations in freshwater tend to increase with increasing agricultural land use (O'Brien & Wehr, 2010; Gabel *et al.*, 2012; Urrea-Clos *et al.*, 2014), and there is a positive relationship between chlorophyll *a* concentration and water nutrients (Mosisch *et al.*, 1999; Dodds *et al.*, 2002; Sabater *et al.*, 2005). The results of this study are consistent with Gabel *et al.* (2012) who found that benthic algal biomass and trophic diatom index were significantly higher in streams lacking agricultural management practices. Further, it appears that nitrogen was a limiting nutrient for algal biomass in ponds but not in streams as indicated by a significant positive relationship between total nitrogen and chlorophyll *a* concentrations in ponds. This confirms findings of other studies (Mosisch *et al.*, 1999; Flecker *et al.*, 2002; Lepori & Robin, 2014; Urrea-Clos *et al.*, 2014). Lepori and Robin (2014) found that nitrogen enrichment in Alpine lakes in France increases algal biomass and alters phytobenthic composition resulting in higher abundance of green algae. Generally, the relationship between water nutrients and chlorophyll *a* concentrations are weaker in streams compared to lakes (Dodds *et al.*, 2002).

Algal biomass could also be affected by other factors, such as canopy cover (Mosisch *et al.*, 2001), pH (Soininen, 2002) and grazers (Hillebrand *et al.*, 2004), and these factors could also be affected by agricultural land use. One explanation for the higher concentrations of chlorophyll *a* found in arable water bodies could be the higher concentrations of nitrogen leading to algal blooms and an increased growth of filamentous green algae especially in ponds. Another explanation could be the low abundance of aquatic snail grazers in arable water bodies (Chapter 2) which might be affected by the use of metaldehyde slug pellets (used as a molluscicide) in arable farming. Biggs *et al.* (2014) reported that there was a regular presence of metaldehyde in stream water in the study catchments in autumn 2012/2013 and 2013/2014 during the application period and low contamination (maximum ranged 1-3 µg/l) was detected.

Benthic diatom communities in freshwater can be affected by anthropogenic activities and changes in land use (Bellinger *et al.*, 2006; Della Bella & Mancini, 2009; Yu & Lin, 2009; Bere & Tundisi, 2011). Researchers have reported different impacts of land use patterns on freshwater diatom community structure. The results of the current study in streams are inconsistent with Yu and Lin (2009) who found an increase in diatom species richness and diversity in subtropical streams associated with increasing agriculture. The results for pond diatoms in the current study are consistent with Della Bella and Mancini (2009), who found that intermediate impacted ponds had significantly higher diatom species than reference and degraded ponds and that degraded ponds had the highest nitrate concentrations.

Higher concentrations of nutrients in water resulting from agriculture can be expected to have important consequences on diatom communities. An increase in diatom diversity, species richness and evenness with the increasing of total phosphorus in agricultural streams was observed in a study by Johnson and Angeler (2014). Although these findings are in contrast with the results of stream diatoms in the current study, it could explain the high species richness, diversity and evenness of diatoms in pastoral ponds which were associated with high concentrations of total phosphorus. In contrast, Jüttner *et al.* (2003) found that diatom diversity increased significantly with increasing nitrate, potassium, chloride and sulphate. This indicate that diatom species are very sensitive to water quality and more likely to respond rapidly to water chemistry (Stevenson & Pan, 1999; Gabel *et al.*, 2012), and any changes in nutrient concentration may alter diatom community composition (Della Bella & Mancini, 2009).

Compositional changes in diatom communities in this study showed different responses of species to the effect of agricultural land use. In streams, principal component analysis of diatom species failed to separate arable and pastoral sites whereas a strong separation of sites between the two types land use have been shown in ponds. Arable ponds were dominated by *Achnanthidium minutissimum*, *Fragilaria capucina* and *Gomphonema truncatum* which are known to be tolerant to low or moderate nutrient enrichment (Kelly *et al.*, 2005). Pastoral ponds had higher abundances of *Planothidium lanceolate*, *Navicula gregaria*, *Navicula minima* and *Nitzschia palea* which are known to be high pollution tolerant species (Bere & Tundisi, 2011). These species are tolerant to phosphorous concentrations up to 1 mg/l in the water (Kelly *et al.*, 2005), and have been recorded in waters that are eutrophic and highly organic polluted (Kelly *et al.*, 2005; Bere & Tundisi, 2011).

In streams, arable and pastoral sites resulted in similar diatom communities and most species are found in both land uses, such as *Achnanthidium minutissimum*, *Navicula lanceolate*, *Navicula gregaria*, *Planothidium lanceolate* and *Nitzschia dissipate*, but they varied in their abundances. Many of these species are motile taxa which are known to be tolerant species, and the high abundance of these species indicates increased sedimentation (Kelly, 2000; Kelly *et al.*, 2005). Higher concentrations of nutrients in agricultural streams could lead to higher occurrence of these tolerant species. For example, *Achnanthidium atomus and Achnanthidium minutissimum* were found to be the most abundant specie in agricultural subtropical streams and they are tolerant to nitrogen (Yu & Lin, 2009). The current findings suggested that the majority of the most abundant species found in streams are tolerant to moderate or high pollution, but they differed in their relative abundances according to the variation in nutrient levels in stream water. It also suggested that phosphorus could be the main driver of changes in the community composition of diatoms in ponds.

The trophic diatom index (TDI) showed different responses to agricultural land uses in ponds and streams. However, as the concentrations of total phosphorous increased, the TDI values increased in both ponds and streams. Several studies suggested a relationship between nutrient levels and TDI (Kelly & Whitton, 1995; Kelly, 1998; Kelly *et al.*, 2008) and TDI was calibrated initially to phosphorous concentrations. However, TDI has been calibrated to both nitrogen and phosphorus (Kelly *et al.*, 2008), and in this study TDI responded only to phosphorus. The current findings are similar to those of Jüttner *et al.* (2003) who observed significant relationships between TDI and phosphorus concentrations in agricultural streams, but no significant relationships were found with nitrate concentrations. In contrast, Gudmundsdottir *et al.* (2013) found that TDI did not relate to either phosphorus or nitrate concentrations and there was no effect of nutrient addition on TDI. Several studies reported an increase in nutrient concentrations associated with increasing agriculture (Yu & Lin, 2009; Gabel *et al.*, 2012; Urrea-Clos *et al.*, 2014) and if TDI does not have an obvious response to nutrients that does not necessarily mean there was no effect on diatom community. To gain a good indication of the trophic status of streams and rivers, it is recommended to take multiple samples during multiple years (Kelly, 1998).

5.5. Conclusion

This study demonstrated that agricultural land use (arable/pastoral) can have a strong effect on algal community in ponds and streams. Both types of water bodies were affected, but the impacts on ponds were more prominent and variable between arable and pastoral land usage. Arable land use seems to cause an increase in algal biomass, especially in ponds. Pastoral land use in ponds was associated with high diatom species richness and species diversity and a strong shift to more tolerant species as indicated by high trophic diatom index (TDI) suggesting eutrophic conditions. In streams, the two types of land use resulted in similar diatom community composition and they were characterized by species that are generally tolerant to pollution. Diatom communities could be a good indicator of the effect of agricultural land use on freshwater habitats.

Chapter 6: The effect of intraspecific interactions on the feeding rate of two common shredder species at two different temperatures

6.1. Introduction

The previous chapters have focused on the effect of agricultural land use on the structure of freshwater invertebrate community (Chapter 3) and algal community (Chapter 5) and on ecosystem functioning measured as leaf decomposition (Chapter 4). This chapter addresses the importance of biotic intraspecific interactions between key shredder species and the subsequent impacts on leaf decomposition. Biotic interactions play an important role in structuring communities and influence many ecosystem processes (Morin, 2011). These interactions may be negative (e.g. competition, predation, parasitism, herbivory) or positive (e.g. mutualism, facilitation, commensalism) and can occur either between individuals of the same species (i.e. intraspecific interactions) or between two or more species (i.e. interspecific interactions) (Holomuzki *et al.*, 2010).

Competition – the interaction of organisms that leads to a change in fitness and growth when the organisms share the same resource – has three major forms: interference competition, exploitation competition or apparent competition (Lang & Benbow, 2013). Interference competition occurs through direct often aggressive interactions, for example when an individual prevents others from entering a favoured habitat or when individuals interfere with the feeding or survival of others (Birch, 1957; Allan & Castillo, 2007; Lang & Benbow, 2013). Exploitation competition is indirect and occurs through the acquisition and depletion of resources, for instance when individuals utilize limited resources and deplete the resources available for others (Allan & Castillo, 2007; Holomuzki *et al.*, 2010; Lang & Benbow, 2013). Apparent competition is also indirect and occurs when individuals that share a common resource are both prey for the same predator. An increase in the abundance of one prey may positively affect the other prey since more sources will be available, or may lead to an increase in predator abundance, which in turn, will decrease the abundance of the other prey (Holomuzki *et al.*, 2010; Lang & Benbow, 2013). The opposite effect could also happen: if the abundance of one prey decreases, it can cause a decline in the abundance of the other prey (Lang & Benbow, 2013).

An important and well-studied ecosystem process in freshwater ecosystems is leaf litter decomposition, in which shredder invertebrates play a key role (Cummins & Klug, 1979; Webster & Benfield, 1986; Graça, 2001). Previous experimental studies have investigated the effect of species loss on decomposition processes (Jonsson & Malmqvist, 2000; Boyero *et al.*, 2007; Reiss *et al.*, 2011; Perkins *et al.*, 2014) and it has been proposed that leaf litter processing in streams responds more to shredder diversity than either resource (i.e. leaf litter) diversity or microbial decomposer diversity (Gessner *et al.*, 2010). Within the same shredder species, it is often assumed that the performance of individuals will decline with increasing density due to an increase in intraspecific competition (Jonsson & Malmqvist, 2003; Boyero & Pearson, 2006).

Boyero and Pearson (2006) found a decrease in decomposition rate per capita and per mg of animal with increased density of four Australian shredder species (three caddisfly species – *Anisocentropus kirramus*, *Lectrides varians* and *Triplectides gonetalus* and one mayfly species – *Atalophlebia* sp.). Jonsson & Malmqvist (2003), investigating the effect of density variation (i.e. 4, 6 and 12 individuals) in three species of detritivorous stoneflies on leaf processing rate, also reported a significant decrease in processing rate (g / individual) with increasing density, but only for one species, *Nemoura picteti*. *Protonemura meyeri* and *Taeniopteryx nebulosi* showed no significant effect of density on processing rate. Similarly, McKie *et al.* (2008), studying the effect of shredder density (i.e. 2, 6, 12, 24 individuals) on leaf processing in microcosm experiments in three different regions (Sweden, Romania and Ireland), also reported interspecific variation in response to changing density. Experiments in each region compared three different detritivores, but the species varied among regions: Sweden – stoneflies *Protonemura meyeri*, *Nemurella pictetii* and *Taeniopteryx nebulosa*; Romania – stonefly

Nemoura fulviceps and caddisflies *Potamophylax rotundipennis* and *Mesophylax impunctatus*; Ireland – caddisflies *Potamophylax cingulatus* and *Halesus radiatus* and isopod *Asellus aquaticus*. Whereas all three stonefly species studied in Sweden exhibited a decrease in leaf processing efficiency (% leaf mass loss/metabolic capacity) with increasing density, only one of the three species studied in Ireland (*P. cingulatus*) and one species studied in Romania (*M. impunctatus*) exhibited a relationship between density and leaf processing efficiency. However, whereas leaf processing efficiency of *P. cingulatus* decreased with increasing density, for *M. impunctatus* it increased with increasing density.

Increased intraspecific densities can be associated with a decline in survival rates as a result of competition for limited sources or increased predation rates (Stachowicz, 2001). However, living within large groups of individuals can offer benefits (i.e. intraspecific facilitation), such as enhance foraging success or collaborative defence against predators (Stachowicz, 2001). Both Jonsson and Malmqvist (2003) and McKie *et al.* (2008) assumed that the strength of interference intraspecific competition is species dependent. Jonsson and Malmqvist (2003) speculated that interspecific variation in activity levels and body size variation influenced interference strength: higher activity increasing encounter rates and larger size differences resulting in more foraging time lost through aggressive interactions. Contrasting activity/aggression levels and intraspecific facilitation mechanisms were also proposed as potential explanations for interspecific variation in interference strength by McKie *et al.* (2008) although they also suggested that other factors such as elevated pathogen load at high densities, interactions between detritivores and microbes and availability of other resources (e.g. faecal pellets) may also be important. Furthermore, groups of individuals may be better in their ability to endure physiological stresses than individuals in isolation (Stachowicz, 2001). However, exploitation competition has been observed in shredder species in a study by Del Arco *et al.* (2015), who found that competition for food resources (intra- and inter-specific) may affect the response of sensitive aquatic invertebrates to the fungicide carbendazim in a more complex way.

Intraspecific interactions may be affected by changes in environmental conditions. It has been demonstrated that the competitive outcomes of closely related species can be determined by specific environmental variables (Holomuzki *et al.*, 2010). For example, the effects of increasing temperature on consumption rates of two predator species *Pterostichus melanarius* and *Poecilus versicolor* and on intraspecific interference competition have been studied by Lang *et al.* (2012), who found that the consumption rate per capita decreased with increasing density of both predator species. They also found that the effects of warming on interference competition varied between predator species: whereas interference competition increased with increasing temperature for *P. melanarius*, it decreased for *P. versicolor* (Lang *et al.*, 2012). In addition, Nilsson-Örtman *et al.* (2014) investigated experimentally the effect of competitive interactions and temperature on growth rates, survival and cohort size structure on the larvae of two damselfly species *Coenagrion armatum* and *C. pulchellum*. They found that the relationship between growth rate and temperature was steeper with competition in *C. armatum* but survival was not affected, whereas in *C. pulchellum* the relationship between growth rate and temperature was unaffected by competition but survival decreased with increasing temperature in the presence of interspecific competition.

It is well known that leaf decomposition and feeding rates of shredders are affected by temperature. Leaf litter decomposition rates were significantly correlated with stream temperatures (Friberg *et al.*, 2009), and the feeding rates of shredders increased with increasing temperature (Maltby *et al.*, 2002; Coulaud *et al.*, 2011; Batista *et al.*, 2012; Boyero *et al.*, 2014). For example, in situ feeding rates were lower in winter than in summer/autumn field deployments and 76% of the variation in shredder feeding rates was accounted for water temperature (Maltby *et al.*, 2002). Moreover, an increase in temperature by one degree might result in an increase of 7.3% in feeding rates (Coulaud *et al.*, 2011).

Previous experimental studies showed that temperature and shredder species richness can have an important impact on leaf decomposition (Boyero *et al.*, 2014; Perkins *et al.*, 2014). It has been suggested that species identity is a main driver of decomposition and that species loss can have similar or greater effect on decomposition than variation in temperature (Boyero *et al.*, 2014). Perkins *et al.* (2014) suggested that higher biodiversity is needed to maintain multiple ecosystem functions across a range of environmental temperatures. However, these studies do not take into account the possible interactive effect of shredder density and temperature on leaf decomposition. Since leaf decomposition is strongly affected by temperature as well as individual density, it is important to understand how intraspecific interactions and changes in temperature influence leaf breakdown.

The aim of this study was to investigate the effect of intraspecific interactions of freshwater shredders on leaf decomposition at two different temperatures. This was achieved by conducting two feeding experiments using three abundance treatments (individual, group of 5 and group of 15) and two different temperatures (high 21°C and low 7°C). The high and low temperatures represent the average maximum temperature in summer and winter in England (Met Office, 2013). The experiments were performed separately with two freshwater crustacean species: *Gammarus pulex* (Amphipoda) and *Asellus aquaticus* (Isopoda). These species were selected because they are known to differ in their intraspecific interactions. *G.pulex* is an aggressive and competitive species, in which cannibalism (intraspecific) and predation (interspecific) have been observed (Willoughby & Sutcliffe, 1976; Dick *et al.*, 1995). In contrast, *A. aquaticus* is regarded as a less aggressive and slow moving species (Blockwell *et al.*, 1998). Both species are widespread in the UK and known to be shredders feed on leaf litter and play an important role in leaf litter decomposition in freshwater ecosystems (Webster & Benfield, 1986; Maltby, 1995; Kunz *et al.*, 2010). They have been used as indicators of water quality; *A. aquaticus* is more tolerant to pollutants than *G. pulex* (Naylor *et al.*, 1990; Maltby, 1995).

The objectives of this study were to: (1) explore the influence of abundance on the per capita leaf mass loss and feeding rate at two different temperatures; and (2) examine how temperature affects intraspecific interactions in each study species. It was expected that: (i) feeding rate per mg of shredder would decrease with increasing abundance, i.e. feeding rate of individuals in isolation would be higher than feeding rate of groups of individuals together; (ii) study species would differ in their response to the effect of intraspecific interactions, which would be stronger in *G. pulex*; and (iii) the effect of intraspecific interactions on feeding rate would be more obvious at high temperature.

6.2. Methods

6.2.1. Leaf discs and fungus culture preparations

The fungus *Cladosporium herbarum* was cultured as described in Section 4.2.4.1. Whole leaf discs were used in experiment with *G pulex* whereas half discs were used in experiment with *A. aquaticus*. A total of 2280 leaf discs were prepared from alder leaves (*Alnus glutinosa*) collected prior to abscission in October 2014 from Endcliffe Park (National Grid Reference: SK 323 858) using methods described in Section 4.2.4.2.

6.2.2. Animal collection and maintenance

Gammarus pulex (Crustacea, Amphipoda) were collected from Crags Stream in Clowne, Derbyshire, United Kingdom (National Grid Reference: SK 497 744) by the same method as described in Section 4.2.4.3. *Asellus aquaticus* (Crustacea, Isopoda) were collected from Rivelin Pond in Sheffield, South Yorkshire, United Kingdom (National Grid Reference: SK 290 872). *A. aquaticus* were collected by slowly sweeping a pond net (1 mm mesh size) near the bottom of the pond to agitate the leaf materials and capture animals within it. The contents of each sample were placed in to a sorting tray and *A. aquaticus* were moved to a bucket containing pond water using forceps.

Each species was transported to the laboratory and allocated to eight 2-L plastic containers containing stream (*G. pulex)* or pond (*A. aquatics)* water (approx. 60 animals/container). Four containers were placed in each of two controlled temperature rooms set at 21 ± 0.5°C and 7 ± 0.5°C and aerated. After 24 hours, stream or pond water was gradually replaced with aerated artificial pond water (APW) (Naylor *et al.*, 1989) and animals were fed with alder leaves conditioned with the fungus *C. herbarum*. All animals were acclimated for 3 days to the experimental temperature (21°C or 7°C) with 12 h light: 12 h dark photoperiod before the start of experiment.

6.2.3. Experimental system

Experiments with *G. pulex* and *A. aquaticus* were performed separately. For each species, the experiment was performed at two temperature treatments (high = 21°C and low = 7°C) and three group sizes: 1 (Individual), 5 (Group 5) and 15 (Group 15) were used. Test vessel size varied with group size: individuals were held in 60-ml glass jars with 40 ml APW whereas groups were held in 500-ml plastic pots with either 150 ml (Group 5) or 300 ml (Group 15) APW (Figure 6.1). Per capita food supply (i.e. *Cladosporium*-conditioned leaf discs) was constant across treatments. Each individual of *G. pulex* received 3 leaf discs (i.e. 3 discs for Individual treatment, 15 discs for Group 5 and 45 discs for Group 15), whereas each *A. aquaticus* received half a leaf disc (i.e. a half disc for Individual treatment, 5 half discs for Group 5 and 15 half discs for Group 15). The amount of leaf material provided to each species was based on the results of preliminary feeding experiments. All leaf discs were rehydrated in artificial pond water before being offered to animals.

For the *G pulex* experiment, there were a total of 75 animals in each treatment which. These were allocated to 75 test vessels for the Individual treatment, 15 test vessels for the Group 5 treatment and five test vessels for Group 15 treatment. Due to the limited availability of *A. aquaticus*, the total number of animals per treatment was 60: 60 test vessels for the Individual treatment, 12 test vessels for Group 5 treatment and five test vessels for Group 15 treatment. A further five test vessels per treatment contained leaf discs and APW only and provided a control of leaf mass loss due to non-feeding activity. All animals and leaf discs were assigned randomly to test vessels which were aerated with small needles. Water levels in test vessels were maintained by refilling with distilled water. Animals were monitored daily for mortality (in all treatments) and moulting (in the Individual treatments only). It was difficult to monitor moulting in the Group treatments. All experiments were terminated after six days, when animals and leaf discs were removed, oven dried at 60° C for 4 days and weighed.

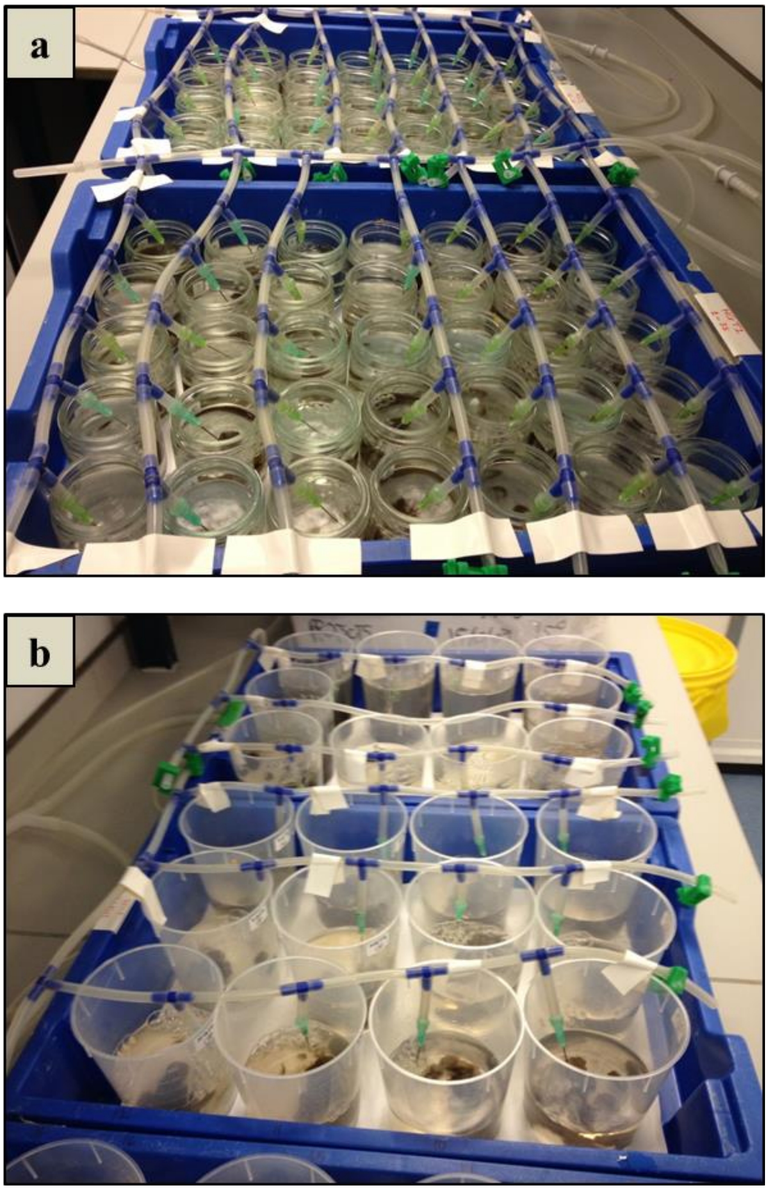

Figure 6.1 A photograph of the test vessels used for (a) individual shredders and (b) groups of shredders in the feeding rate experiments.

6.2.4. Data analysis

Feeding rate (FR, mg dry food weight/ mg dry animal weight/ day) was calculated using the following equation (Maltby *et al.*, 2002):

$$FR = \frac{(L_i \times C) - L_f}{W \times t} \qquad \text{Equation 6.1}$$

where L_i is initial dry weight of leaf discs (mg), L_f is final dry weight of leaf discs (mg), W is dry weight of the animal (mg), t is experiment period (6 days) and C is correction factor for non-consumptive mass loss and given by the mean of the quotient of final to initial dry weight of control leaf discs.

Per capita leaf mass loss (per capita LML, mg) was calculated by subtracting the final leaf mass from the initial leaf mass multiplied by the correction factor for non-consumptive mass loss (C) then dividing by the number of animals alive at the end of the experiment.

$$LML = \frac{(L_i \times C) - L_f}{No.\ of\ alive\ animals} \qquad \text{Equation 6.2}$$

Two-way ANOVAs, followed by Tukey’s post hoc multiple comparison test, were used to analyse the effect of abundance and temperature on the feeding rate, per capita leaf mass loss, individual body mass and survival rate for each of the study species. In order to remove the influence of sample size differences across treatments, data were pooled to give 5 replicates per treatment each comprising a total of either 15 (*G. pulex*) or 12 (*A. aquaticus*) animals. Normality and homogeneity of variance were checked using Shapiro-Wilk and Levene's tests. FR and LML were natural logarithm transformed and survival rate was arcsine square root transformed prior to analysis. Statistical analyses were carried out using R (R Core Team, 2013). In R, the package ggplot2 (Wickham, 2009) was used for producing graphics.

6.3. Results

6.3.1. Survival rate

The survival rate for both species was greater than 78.7% but varied by treatment (Figure 6.2). In the Individual treatment, there was no mortality for *G. pulex* at either temperatures and for *A. aquaticus* mortality was only observed at high temperature (96.7%). The survival of *G. pulex* was significantly lower at larger group sizes and higher temperatures and there was a significant group size by temperature interaction; the negative effect of group size was greater at the higher temperature (Table 6.1, Figure 6.2a). In contrast, only temperature had a significant effect on the survival rate of *A. aquaticus*; the higher the temperature, the lower the survival rate (Table 6.1, Figure 6.2b).

The number of moulting animals in the Individual treatment increased with increasing temperature for both species. In the *G. pulex* experiment, the moulted gammarids were 17 individuals at high temperature compared to 9 individuals at low temperature. In the *A. aquaticus* experiment, there were 14 moulted individuals at high temperature compared to 6 moulted individuals at low temperature.

6.3.2. Per capita leaf mass loss

In the *G. pulex* experiment, leaf mass loss in the Individual treatment ranged from 9.07 to 11.33 mg at high temperature compared to 4.88 to 6.15 mg at low temperature (Figure 6.3a). The per capita leaf mass loss was significantly greater at higher temperature, but there was no significant effect of abundance on per capita leaf mass loss and no significant interaction between abundance and temperature (Table 6.1, Figure 6.3a). In the *A. aquaticus* experiment, leaf mass loss in the Individual treatment ranged from 0.28 to 0.55 mg at high temperature and from 0.02 to 0.09 mg at low temperature (Figure 6.3b). The per capita leaf mass loss was significantly greater at larger group sizes and higher temperatures, but there was no significant interaction between them (Table 6.1, Figure 6.3b). The per capita leaf loss in the Individual treatment was significantly lower than Group 5 and Group 15 treatments (Figure 6.3b, Tukey's test: $p < 0.01$, $p < 0.001$, respectively). Group 15 treatment of *A.aquaticus* showed the greatest effect on per capita leaf mass loss at both temperatures, Group 5 was intermediate, whereas Individual had the smallest effect (Figure 6.3b) and the effect of abundance was greater at higher temperature.

Table 6.1 Two-way ANOVAs the effect of abundance and temperature (Temp) on survival rate (%), per capita leaf mass loss (LML), feeding rate (FR) and individual body mass (BM) of *G. pulex* and *A. aquaticus* experiments. Significant differences are highlighted in bold ($p < 0.05$).

Factor		**Survival rate (%)**		**per capita LML**		**FR**		**Individual BM**	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
***G. pulex* experiment**									
Abundance	2	13.58	**< 0.001**	0.27	0.77	3.05	0.066	4.38	**< 0.05**
Temp	1	24.18	**< 0.001**	172.7	**< 0.001**	267.8	**< 0.001**	8.36	**< 0.01**
Abundance x Temp	2	7.13	**< 0.01**	0.27	0.77	0.40	0.67	0.81	0.46
Residuals	24								
***A.aquaticus* experiment**									
Abundance	2	1.73	0.20	20.43	**< 0.001**	1.64	0.21	2.07	0.15
Temp	1	11.16	**< 0.01**	125.6	**< 0.001**	61.7	**< 0.001**	1.37	0.25
Abundance x Temp	2	0.65	0.53	2.10	0.15	1.34	0.28	0.31	0.74
Residuals	24								

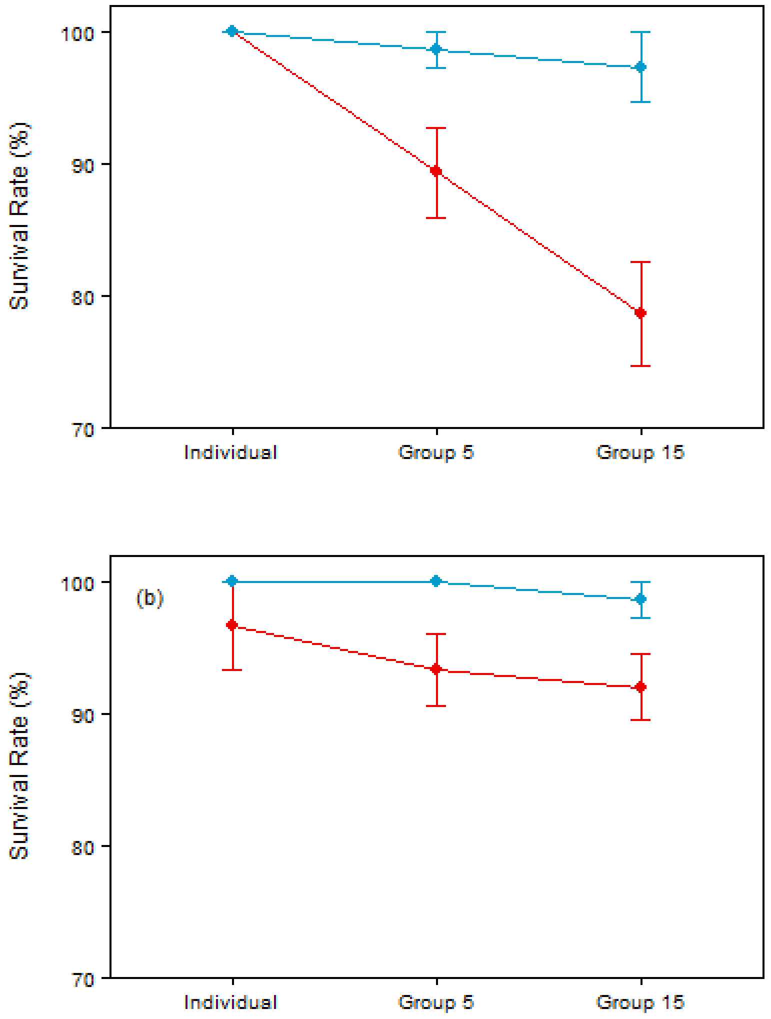

Figure 6.2 Mean (± SE) survival rate of (a) *G. pulex* and (b) *A. aquaticus* at high temperature (red) and low temperature (blue).

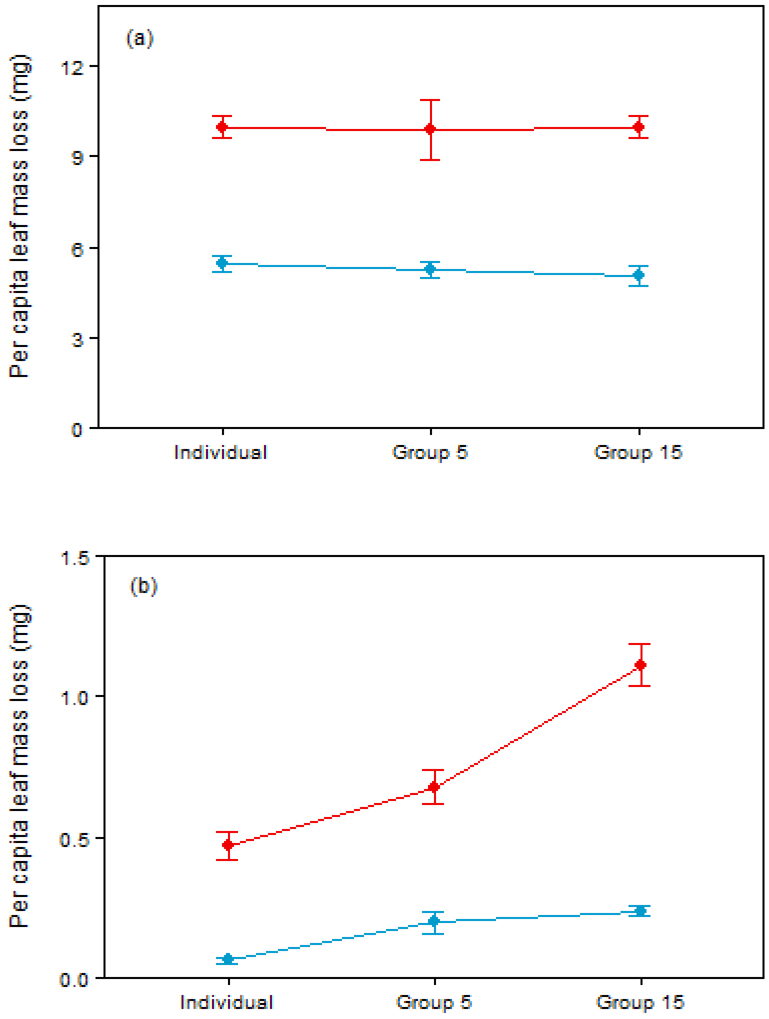

Figure 6.3 Mean (± SE) per capita leaf mass loss (mg dry weight) of (a) *G. pulex* and (b) *A. aquaticus* at high temperature (red) and low temperature (blue).

6.3.3. Body mass

Body mass of experimental animals is presented in Table 6.2 and Figure 6.4. There was significant variation in body mass across treatments with the mean body mass of *G. pulex* allocated to low temperature treatments being larger than those allocated to high temperature treatments. This difference was most marked for the Individual treatment compared to Group 15 treatment (Table 6.1, Tukey's test: $p < 0.05$, Figure 6.4a). In contrast, mean body mass of *A. aquaticus* was lower at low temperature and increased with increasing group size, although these differences were not statistically significant (Table 6.1, Figure 6.4b).

Table 6.2 Mean ± SE of individual body mass (mg dry weight) of *G. pulex* and *A .aquaticus* in the feeding rate experiments.

Temperature	Abundance treatment	*G. pulex*	*A. aquaticus*
High	Individual	6.95 ± 0.17	6.26 ± 0.49
	Group 5	7.61 ± 0.18	7.66 ± 0.74
	Group 15	7.82 ± 0.31	7.96 ± 1.09
Low	Individual	7.80 ± 0.31	5.70 ± 0.88
	Group 5	7.92 ± 0.13	6.02 ± 0.80
	Group 15	8.27 ± 0.21	7.62 ± 1.15

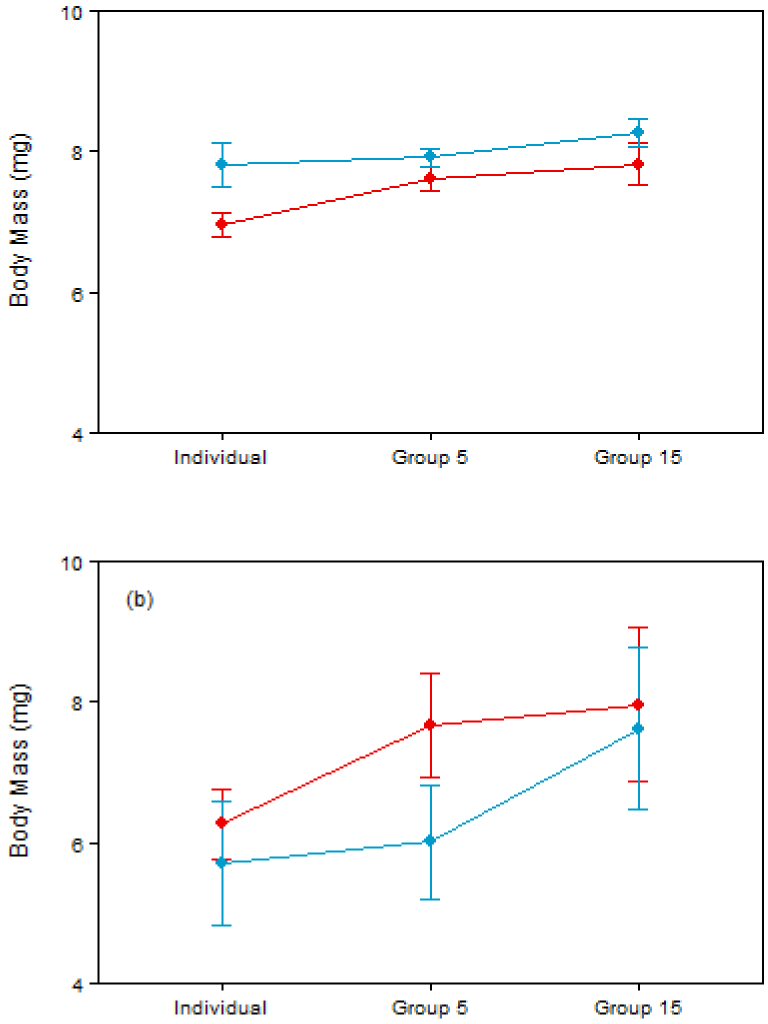

Figure 6.4 Mean (± SE) individual body mass (mg dry weight) of (a) *G. pulex* and (b) *A. aquaticus* at high temperature (red) and low temperature (blue).

6.3.4. Feeding rate

Individual feeding rate of *G. pulex* ranged from 0.21 to 0.26 mg/mg/day at high temperature, and from 0.11 to 0.12 mg/mg/day at low temperature (Figure 6.5a). The feeding rate of *G. pulex* was significantly greater at high temperature (Table 6.1), and decreased with increasing abundance; being higher at Individual than Group 15 treatments (Figure 6.5a, Tukey's test: *p* = 0.058). However, the effect of abundance on feeding rate of *G. pulex* was marginally non-significant and there was no significant interaction between abundance and temperature (Table

6.1). In the *A. aquaticus* experiment, the individual feeding rate ranged from 0.007 to 0.036 mg/mg/day at high temperature and from 0.002 to 0.004 mg/mg/day at low temperature (Figure 6.5b). The feeding rate of *A. aquaticus* was significantly greater at high temperature (Table 6.1). It also varied among treatments; the feeding rate in Group 5 was the lowest at high temperature but the greatest at low temperature (Figure 6.5b). However, there was no significant effect of abundance on the feeding rate *A. aquaticus* and also no significant interaction between abundance and temperature (Table 6.1).

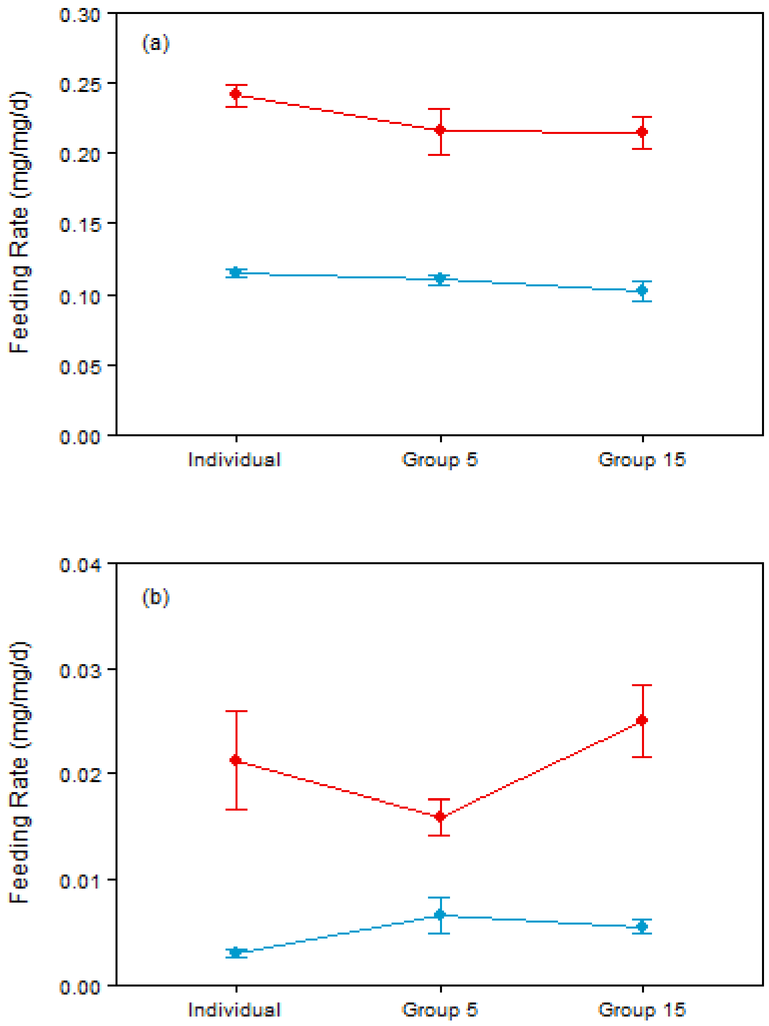

Figure 6.5 Mean (± SE) feeding rate of (a) *G. pulex* and (b) *A. aquaticus* at high temperature (red) and low temperature (blue).

6.4. Discussion

The aim of this study was to investigate the effect of shredder intraspecific interactions on leaf decomposition at two different temperatures. The feeding rate of the two freshwater shredder species, *G. pulex* and *A. aquaticus*, was examined at 21°C (high) and 7°C (low) representing the UK average maximum summer and winter temperatures. Consistent with the study expectations, temperature had significant effects on survival rates, per capita leaf mass loss and feeding rates of both species. Contrary to expectations, increasing abundance had a significant effect only on per capita leaf mass loss of *A. aquaticus* and the survival rate of *G. pulex*.

The increase in feeding rate and leaf mass loss with increasing temperature observed in the current study is consistent with previous observations (Webster & Benfield, 1986; Maltby *et al.*, 2002; Coulaud *et al.*, 2011; Boyero *et al.*, 2014). For example, Boyero *et al.* (2014) observed an increase in per capita leaf decomposition rates of three caddisfly species as a result of an increase in water temperature of 5°C. Similarly, Coulaud *et al.* (2011) found that an increase in temperature by one degree might result in an increase of 7.3% in feeding rates of *Gammarus fossarum*. Furthermore, these findings also support the general metabolic theory that metabolic rate and other biological rates and activities increase with increasing temperature (Honek, 1997; Gillooly *et al.*, 2001; Brown *et al.*, 2004).

Contrary to expectations, the per capita leaf mass loss of *G. pulex* did not change with increasing abundance. When variation in body mass was accounted for, the mass specific feeding rate of *G. pulex* decreased with increasing abundance, although this was marginally non-significant. This finding was consistent with previous studies (Boyero & Pearson, 2006) who found a decrease in decomposition rate per mg of animal with increased density of four Australian shredder species.

The combination of increasing abundance and increasing temperature had a strong effect on the survival rate of *G. pulex*. Cannibalism was observed during the *G. pulex* experiment at high temperature and it was more prominent in the largest group size (Group 15). Cannibalism has been reported previously in *Gammarus* spp. (Willoughby & Sutcliffe, 1976; Dick *et al.*, 1995; MacNeil *et al.*, 1997) with moulting individuals being particularly vulnerable. Moulting may allow intraspecific predation of equal sized or even larger individuals (MacNeil *et al.*, 1997). It was observed that the number of moulted gammarids was greater at the higher temperature (Individual treatments: 17 moulted at high temperature compared to 9 moulted at low temperature). This result confirmed findings of a study by Pöckl (1992) who found that the number of moults of *Gammarus fossarum* and *G. roeseli* increased with increasing temperature.

Contrary to expectations, the per capita leaf mass loss of *A. aquaticus* increased with increasing abundance. When variation in body mass was accounted for, the effect of abundance was not significant on the mass specific feeding rate of *A. aquaticus*. This result is in contrast with the finding by McKie *et al.* (2008) who found a decrease in leaf processing efficiency of *A. aquaticus* with increasing density although this relationship was not statistically significant. Further, there was no significant effect of increasing abundance on the survival rate of *A. aquaticus*. *A. aquaticus* is known to be a less aggressive and slow moving species (Blockwell *et al.*, 1998) and lack of cannibalism was been observed in the current study. In addition, the feeding technique differs between the two experimental species: whereas *A. aquaticus* scrapes leaf surface and consumes fungal mycelia, *G. pulex* bites leaves and feeds on whole leaf materials (Graça *et al.*, 1993). This feeding technique of *A. aquaticus* could help in collecting more food in high density.

Interference interactions between individuals can be direct encounters, related to variation in activity and aggression levels (Jonsson & Malmqvist, 2003; Boyero & Pearson, 2006). In contrast, the positive effect of density on leaf processing in some species might reflect intraspecific facilitation (McKie *et al.*, 2008), in which invertebrates collect more resources in aggregation (Sommer, 1992; Heard & Buchanan, 1998). The results of the current study confirmed that abundance can affect feeding rate of shredders, but species markedly differed in their responses (Jonsson & Malmqvist, 2003; McKie *et al.*, 2008).The contrasting responses of the study species indicated the principle differences of intraspecific interactions for the study species, which was associated in *G. pulex* with aggressive activity and cannibalism (MacNeil *et al.*, 1997; Jonsson & Malmqvist, 2003; Boyero & Pearson, 2006), while in *A. aquaticus* might reflect intraspecific facilitation (McKie *et al.*, 2008). However, the effect of density on leaf processing might result from indirect mechanisms, such as increased loads of pathogen or toxin from individuals at high density assemblage (Kohler & Hoiland, 2001), elevated contamination from excretion or decrease in oxygen concentration (O'Connor, 1993), interaction between microbes and invertebrates (Förster *et al.*, 2006), and alternative resource availability, e.g. faecal particles (Rossi & Vitagliano-Tadini, 1978). These indirect mechanisms are unlikely to be the case in the current study.

It is often assumed that there is a relationship between the number of species, their behaviour patterns and intra- and inter-specific interactions, which affects processing rates (Boyero & Pearson, 2006). Even at low density, individuals may have many encounters which can impede leaf processing (Boyero & Pearson, 2006). The intraspecific interactions of key species may affect their feeding rate and can thus affect ecosystem functioning and associated ecosystem services. Moreover, the combination of biotic interactions and environmental factors may alter the response of populations and communities to environmental variables in a complex way (Lang *et al.*, 2012). Laboratory studies tend to focus on the effect of certain stressors whereas, in natural systems, freshwater organisms are exposed to multiple stressors and involved in interactions with other species and hence they may respond differently from in the laboratory (Duarte *et al.*, 2008; Fernandes *et al.*, 2009).

6.5. Conclusion

This study demonstrated that intraspecific interactions can affect leaf mass loss and that this effect will be more prominent with increasing temperature. In *G. pulex*, competitive interaction (interference) and cannibalism reduced survival rate, whereas in *A. aquaticus*, per capita leaf mass loss increased in the presence of intraspecific interaction (facilitation). The contrasting responses of the study species suggested that the effect of intraspecific interactions on leaf breakdown depend on shredder species and temperature. The increase in temperature, as a result of climate change, is expected to influence organisms and their role in ecological processes in ecosystems.

Chapter 7: General discussion

Sustainable intensification of food production is essential for future global food security (Tilman *et al.*, 2002; Foley *et al.*, 2011; Foresight, 2011; Godfray & Garnett, 2014). However, intensive agricultural production can have adverse environmental impacts; and freshwater ecosystems are particularly vulnerable to chemical and physical stressors resulting from agricultural practices (Allan, 2004; Dudgeon *et al.*, 2006). Freshwater ecosystems in agricultural landscapes may enhance human wellbeing by, for example, alleviating floods, removing and detoxifying pollutants and regulating soil loss (Firbank *et al.*, 2011). However, agricultural practices that change the structure and functioning of freshwater communities may reduce these wellbeing benefits known as ecosystem services (Dudgeon, 2010; UK National Ecosystem Assessment, 2011).

The central aim of this thesis was to investigate the effects of agricultural land use (arable and pastoral) on the community structure and functioning of ponds and streams. The study was based on 12 ponds and 12 streams which were part of the Water Friendly Farming project located at Loddington in Leicestershire, UK (Biggs *et al.*, 2014) (Chapter 2). The study had four main objectives: (1) investigate the impact of two types agricultural land use, arable and pastoral, on aquatic macroinvertebrate communities in ponds and streams (Chapter 3); (2) explore how different types of agricultural land use influence leaf litter decomposition in ponds and streams (Chapter 4); (3) compare algal communities in ponds and streams located in different agricultural land use (Chapter 5); and (4) explore the relative importance of intraspecific interactions between key shredder species and the subsequent impacts on leaf decomposition at two different temperatures (Chapter 6). This chapter summarizes the key findings of this study, provides a synthesis of the results and considers the implications for ecosystem service delivery and the sustainable intensification of agriculture.

7.1. Main findings

7.1.1. Aquatic macroinvertebrate communities in agricultural ponds and streams

Whereas the total number and taxonomic richness of invertebrates were significantly lower in arable than pastoral streams, there was no significant effect of land use on either the number or richness of invertebrates in ponds. Arable streams had significantly higher abundance of Oligochaeta and lower abundance of Plecoptera than pastoral streams. Diptera (mainly Chironomidae) were the dominant group in both types of pond, although their relative abundances were significantly higher in arable ponds. In terms of functional feeding groups, shredders and scrapers were more abundant in pastoral than arable water bodies. Macroinvertebrate community composition differed significantly between arable and pastoral streams, but not between arable and pastoral ponds.

In a study comparing stream macroinvertebrate communities in reference sites and two types of agricultural practices in the USA, corn fields and dairy cattle pasture, Kyriakeas and Watzin (2006) found that both types of agricultural sites were impacted compared to reference sites and sites in dairy pasture were more impacted than sites in corn fields. This does not appear to be the case in the current study where invertebrate communities seem to be more impacted in arable streams. However, whereas cattle had unrestricted access to streams in the study by Kyriakeas and Watzin (2006), in the current study streams were either fenced or had riparian buffers that restricted direct access by livestock. It has been demonstrated that cattle have both physical (disturbance/soil erosion) and chemical (organic pollution) impact on water quality (Dolédec *et al.*, 2006). They found that fine sediment percentage and dissolved reactive phosphorus were six fold higher in the most intensive grassland streams.

The higher abundances of dipterans and oligochaetes in arable ponds and streams may be indicative of the increased abundance of fine, organic rich sediments in these sites (Goodnight, 1973; Kyriakeas & Watzin, 2006). Oligochaetes and Chironomidae are generally pollution tolerant whereas Plecoptera are generally pollution sensitive (Goodnight, 1973; Myslinski &

Ginsburg, 1977). The increased abundance of oligochaetes and decreased abundance of Plecoptera may therefore be an indication of poorer water quality in arable streams.

7.1.2. Leaf litter decomposition in agricultural ponds and streams

Leaf litter processing was higher in arable than pastoral ponds, but there was no significant difference between total (invertebrate plus microbial) and microbial leaf processing, suggesting that feeding by invertebrate shredders played a limited role in leaf decomposition in ponds. The importance of invertebrate feeding by shredder invertebrates was determined by the difference in mass loss between coarse and fine mesh bags. Chironomidae were present in both fine and coarse mesh bags deployed in ponds. Although they generally feed on fine particulate matter, they do have a role in leaf decomposition (Callisto *et al.*, 2007; da Silveira *et al.*, 2013). The palatability of leaf material to invertebrates is a function of the microbial community colonizing the leaf surface and its ability to decompose leaf material (Barlocher, 1985; Gessner *et al.*, 2007). Microbial biomass and decomposition of leaf material is enhanced in nutrient-enriched waters (Gulis & Suberkropp, 2003) and this may explain the higher leaf litter processing in arable ponds.

Leaf litter processing in streams did not differ between the two types of land use, which is similar to the findings of Hagen *et al.* (2006) and Magbanua *et al.* (2010). However, there was a significant effect of shredder feeding on leaf breakdown; leaf breakdown increasing with increasing shredder abundance. This confirmed findings of other studies (Bergfur *et al.*, 2007; Piscart *et al.*, 2009; Menéndez *et al.*, 2011; Piscart *et al.*, 2011).

The feeding rate of *Gammarus pulex*, an important shredding invertebrate, was lower in arable than pastoral streams, but this difference was not statistically significant. *Gammarus* feeding rate was negatively correlated with ammonia concentration and positively correlated with nitrate concentration. This is consistent with previous studies that found no significant difference in the feeding rates of *G. pulex* deployed in streams located in an agricultural catchment (Crane *et al.*, 1995) and a negative effect of ammonia on *Gammarus* feeding rate

(Maltby, 1995) and energy intake (Maltby *et al.*, 1990). It is possible that a 'negative' effect of ammonia on feeding rate was compensated for by a 'positive' effect of nitrate on microbially-induced changes in food quality and hence feeding rate (Gulis & Suberkropp, 2003).

7.1.3. Freshwater algal communities in agricultural ponds and streams

The total algal biomass (i.e. chlorophyll *a* concentration) was higher in arable water bodies than pastoral water bodies, but these differences were only statistically significant for ponds. Diatom community composition was significantly different between arable and pastoral ponds. Arable ponds had lower diatom species richness, diversity and evenness than pastoral ponds, and also had higher abundances of *Achnanthidium minutissimum*, *Fragilaria capucina*, *Synedra acus*, *Cyclotella sp.* and *Gomphonema truncatum*. In contrast, land use had no significant effect on the structure or composition of diatom communities in streams. The trophic diatom index (TDI), a measure of trophic status in streams and rivers (Kelly *et al.*, 2008), was significantly higher in pastoral than arable ponds but there was no significant difference between arable and pastoral streams.

In the current study, arable streams had elevated concentrations of nitrogen and phosphorus whereas arable ponds had elevated concentrations of nitrogen and pastoral ponds had elevated concentrations of phosphorus. It appears that nitrogen was a limiting nutrient for algal biomass in ponds but not in streams as indicated by a significant positive relationship between total nitrogen and chlorophyll *a* concentrations in ponds. This confirms findings of other studies (Mosisch *et al.*, 1999; Flecker *et al.*, 2002; Lepori & Robin, 2014; Urrea-Clos *et al.*, 2014).

Diatom species are very sensitive to water quality and likely to respond rapidly to water chemistry (Stevenson & Pan, 1999; Gabel *et al.*, 2012), and any changes in nutrient concentration may alter diatom composition (Della Bella & Mancini, 2009). The results of the current study are in contrast with Johnson and Angeler (2014), who found that increased total phosphorus in agricultural streams was associated with a significant increase in diatom species richness, diatom diversity and evenness. In ponds, the findings of the current study suggest that phosphorus may have been responsible for a change in diatom composition between the two types of land use.

In the present study, although there was no effect of land use on either algal biomass or leaf decomposition in streams (i.e. stream energy inputs), using different types of agricultural land use was associated with differences in stream invertebrate community structure and composition. These results are consistent with Jinggut *et al.* (2012) who found that although farming had an effect on the structure of stream invertebrates, it did not affect stream functioning (i.e. leaf breakdown). Similarly, Magbanua *et al.* (2010) found that invertebrate taxonomic and trait measures responded to grassland farming intensity (conventional, integrated management and organic) but measures of ecosystem functioning (i.e. leaf decomposition and algal accrual) were less sensitive to farming intensity. In contrast, Gabel *et al.* (2012), investigating the effectiveness of agricultural management practices (i.e. riparian plantation, stream bank fencing, improved manure storage and barnyard improvements) on stream invertebrates and diatoms, found that management practices had no effect on invertebrate taxa richness and diversity but did reduce benthic algal biomass and the trophic diatom index. The authors interpreted this as evidence that diatoms were more sensitive to moderate changes in the concentrations of nutrients, conductivity and pH observed in streams lacking management practices.

7.1.4. Intraspecific interactions and the feeding rate of key shredder species at two different temperatures

Increasing intraspecific interactions (i.e. density) significantly increased per capita leaf mass loss by *Asellus aquaticus* and significantly reduced the survival rate of *Gammarus pulex*. This result is in contrast with the finding by McKie *et al.* (2008) who found a decrease in leaf processing efficiency of *A. aquaticus* with increasing density, although this relationship was not statistically significant. The current study suggested that the competitive interaction (interference) and cannibalism of *G. pulex* decreased survival rate, whereas the presence of intraspecific interaction (facilitation) in *A. aquaticus* increased per capita leaf mass loss. Interference interactions between individuals can be direct encounters, related to variation in activity and aggression levels (Jonsson & Malmqvist, 2003; Boyero & Pearson, 2006). Cannibalism has been reported previously in *Gammarus* spp. (Willoughby & Sutcliffe, 1976; Dick *et al.*, 1995; MacNeil *et al.*, 1997) whereas *A. aquaticus* is known to be a less aggressive and slow moving species (Blockwell *et al.*, 1998). In contrast, the positive effect of density on leaf processing in some species might reflect intraspecific facilitation, in which invertebrates collect more resources in aggregation (Sommer, 1992; Heard & Buchanan, 1998).

Temperature also had a significant effect on survival rates, per capita leaf mass loss and feeding rates of both species. At higher temperature, the per capita leaf mass loss and feeding rates were greater whereas the survival rates were lower. The increase in feeding rate and leaf mass loss with increasing temperature observed in the current study is consistent with previous observations (Webster & Benfield, 1986; Maltby *et al.*, 2002; Coulaud *et al.*, 2011).

7.2. Synthesis

Increased agricultural land use has resulted in degradation of freshwater habitats associated with high nutrient concentrations, sedimentation and pesticide use (Haygarth & Jarvis, 2002; Foley *et al.*, 2005; Moss, 2008) and therefore affecting aquatic biodiversity (Dudgeon *et al.*, 2006). Arable fields are expected to receive generally more fertilizers and pesticides than improved grasslands (Firbank *et al.*, 2011). Water chemistry monitoring data revealed that arable streams in the study catchments generally had higher concentrations of total nitrogen, total phosphorous, nitrate and nitrite than pastoral streams. Additionally, arable ponds had high concentrations of total nitrogen and nitrate while pastoral ponds were mainly associated with high concentrations of total phosphorus and ammonia and low concentrations of dissolved oxygen. Phosphorus loads from grassland can be high when manure is applied or there is intensive livestock grazing (Watson & Foy, 2001).

Nutrient enrichment is one of the main stressors for aquatic communities (Dudgeon *et al.*, 2006) and therefore for trophic interaction. Increased nutrients could result in an increase in algal abundance (Francoeur, 2001; Urrea-Clos *et al.*, 2014), which has the potential to increase abundance of grazing invertebrates (Hillebrand *et al.*, 2004). In some situations, nutrient enrichment might cause changes in algal composition and increase inedible species (e.g. filamentous green algae or cyanobacteria) (Dodds, 2006; Gudmundsdottir *et al.*, 2013), and this may affect food sources available to grazers (Dodds, 2006). In this study, the higher concentrations of nitrogen in arable ponds and streams may have been responsible for a higher total algal biomass (i.e. concentrations of chlorophyll *a*) leading to an increased growth of filamentous green algae which was observed in arable sites. Further, an increase in phosphorus concentration associated with pastoral land use in ponds may potentially affect diatom composition through shifting to more tolerant diatom species. It has been reported that the relationship between water nutrients and chlorophyll *a* concentrations are stronger in lakes compared to streams (Dodds *et al.*, 2002).

Nutrient availability affects algal abundance and composition (Francoeur, 2001), which in turn affects grazer abundance (Hillebrand *et al.*, 2004). An increase in algal abundance, without a shift in community composition, may result in more food for grazers and hence an increase in grazer abundance (Hillebrand, 2002) (Figure 7.1, arrow 1). In contrast, a change in algal composition towards less edible species may result in reduced food availability and a decrease in grazer abundance (Holomuzki & Biggs, 2006) (Figure 7.1, arrow 2). Nutrient enrichment can also cause an increase in the macrophyte biomass (Brock *et al.*, 1995) which may result in more habitats provided for grazers and hence an increase in their abundance (Figure 7.1, arrow 3). However, grazer abundance is also influenced by the direct effects of other environmental stressors such as pesticides. Snails and insects were important grazers in the study sites and these are potentially at risk from molluscicides (applied to control slugs) and insecticides (Van den Brink *et al.*, 2009). Biggs *et al.* (2014) detected metaldehyde (used in slug pellets) in stream water in the study catchments in autumn 2012/2013 and 2013/2014, and metaladehyde has been reported to be toxic to the freshwater snail *Lymnaea stagnalis* (Mills *et al.*, 1990; Mills *et al.*, 1992).

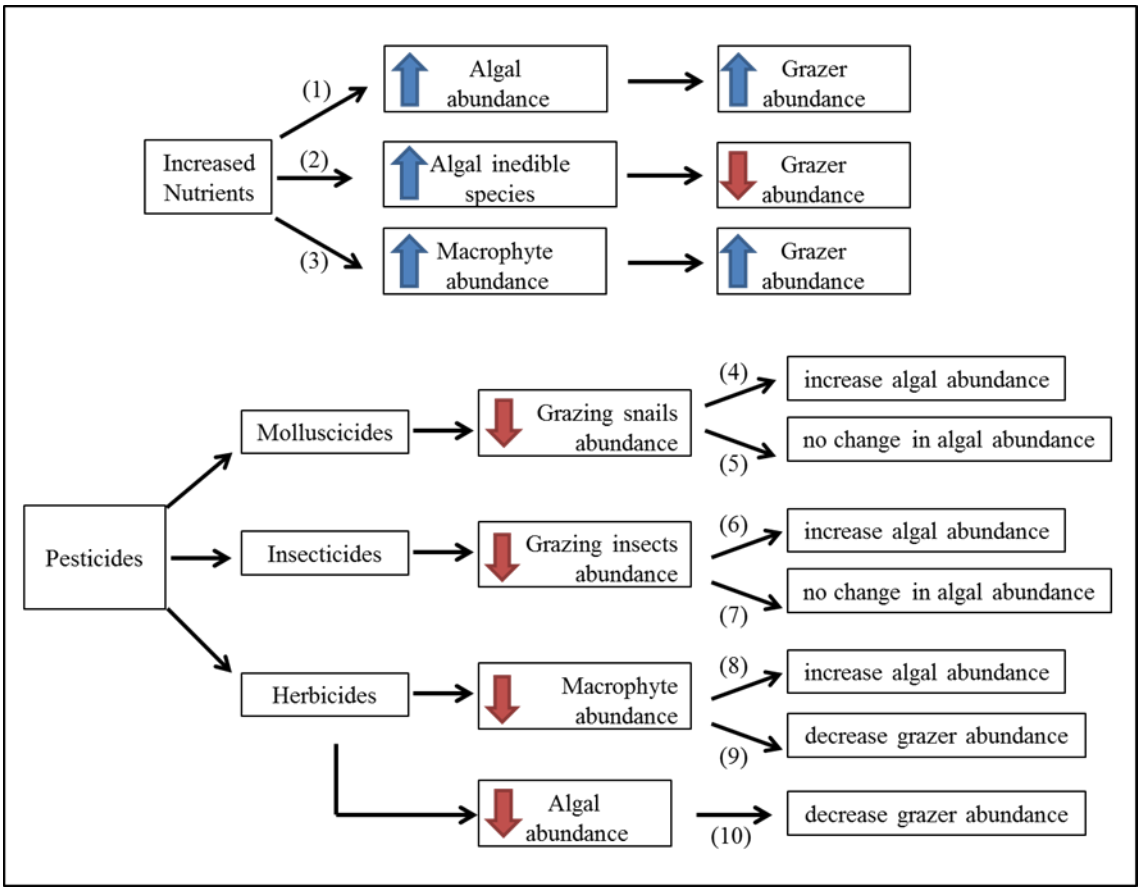

Figure 7.1 Schematic of possible effects of agricultural land use (mainly nutrients and pesticides) on a trophic interaction in freshwater ecosystems. Blue arrows indicate an increase; red arrows indicate a decrease and numbered arrows are explained in the text.

A decrease in grazing snails may cause an increase in primary producers (Holomuzki & Biggs, 2006) (Figure 7.1, arrow 4). Alternatively, the reduction in snails may reduce competition with grazing insects resulting in no net change in algal abundance (Holomuzki *et al.*, 2010) (Figure 7.1, arrow 5). Similarly, insecticides may reduce the abundance of insect grazers resulting in either an increase in algal abundance (Hillebrand, 2002) (Figure 7.1, arrow 6) or no change in algal abundance (Holomuzki & Biggs, 2006) (Figure 7.1, arrow 7). Furthermore, herbicides may reduce the abundances of macrophytes (Van den Brink *et al.*, 1997), which in turn may increase algal abundance as a result of a competition with macrophytes (Figure 7.1, arrow 8) or may decrease grazer abundance (Holomuzki & Biggs, 2006) as a result of habitat loss (Figure 7.1, arrow 9). Additionally, herbicides can also directly affect algal communities and cause a decrease in algal abundance (Van den Brink *et al.*, 1997) and thus resulting in a decrease in grazer abundance (Figure 7.1, arrow 10).

The results from this study are consistent with the hypothesis that increased nutrients (mainly nitrogen) promoted an increase in the abundance of less edible algae and reduced the relative abundance of grazer (snails) populations in arable ponds and streams. The presence of metaldehdye may also have reduced snail abundance in arable ponds and streams and the potential combined effects of nutrients and molluscicides on algal abundance are consistent with the increased algal abundance observed in this study.

Moderate nutrient concentrations can stimulate fungal biomass (Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006), which in turn can increase microbial decomposition (Gessner *et al.*, 2007) and accelerate leaf litter breakdown (Webster & Benfield, 1986) (Figure 7.2, arrow 1). However, high nutrient concentrations can decrease aquatic hyphomycete species richness (Lecerf & Chauvet, 2008). In addition, increased nutrient concentrations can cause a decline in dissolved oxygen concentrations leading to hypoxic conditions and an elevation in ammonia concentrations that may affect shredding invertebrates and other aquatic organisms (Suter & Cormier, 2015) thereby reducing leaf decomposition (Figure 7.2, arrow 2). Pesticides may reduce leaf breakdown either by affecting aquatic microbes (Maltby *et al.*, 2009; McMahon *et al.*, 2012; Dimitrov *et al.*, 2014) or by affecting shredding invertebrates (Maltby *et al.*, 2005; Schäfer *et al.*, 2007; Van den Brink *et al.*, 2009) (Figure 7.2, arrow 4).

The higher leaf litter decomposition in arable ponds observed in this study is consistent with the hypothesis that higher concentrations of nutrients (nitrate) enhanced leaf decomposition in arable ponds and with the hypothesis that high ammonia concentrations and low dissolved oxygen limited the functioning of shredders in pastoral ponds. In streams, the higher leaf decomposition in coarse leaf bags is promoted mainly by shredding invertebrates which were more abundant in pastoral streams. The result of this study is consistent with the hypothesis that the application of insecticides in arable fields may have reduced shredder abundance in arable streams.

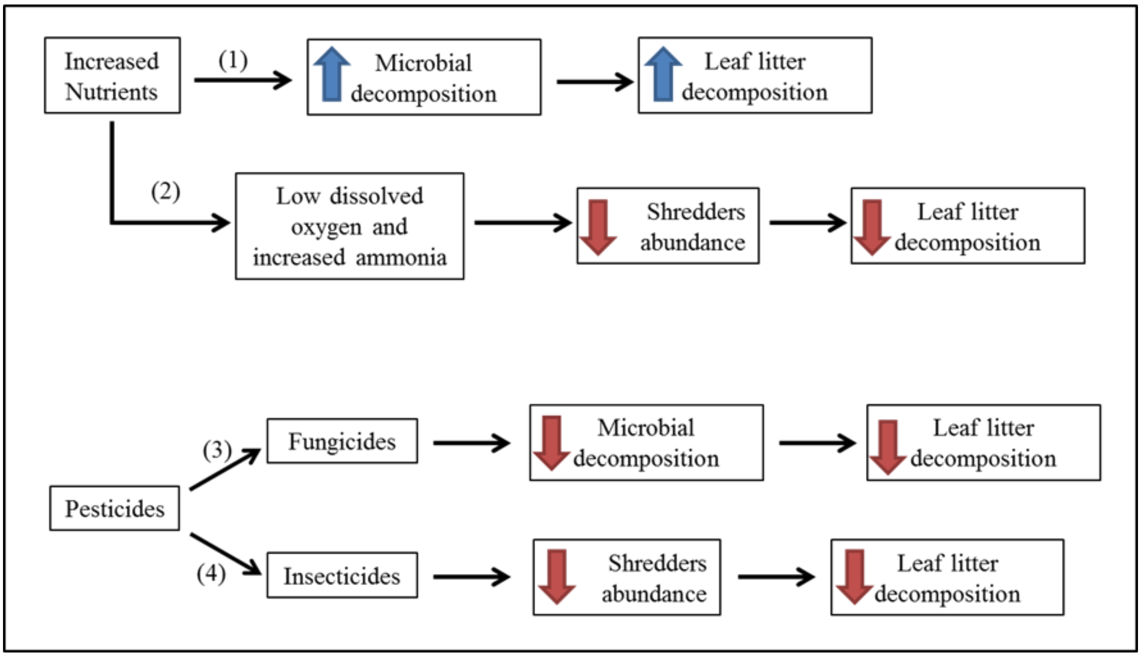

Figure 7.2 Schematic of possible effects of agricultural land use (mainly nutrients and pesticides) on the ecological process (leaf litter decomposition). Blue arrows indicate an increase; red arrows indicate a decrease and numbered arrows are explained in the text.

The results of the current study suggest that nutrients may play a key role in determining the structure and functioning of freshwater communities in agricultural landscapes. There is considerable evidence in the literature that high concentrations of nutrients have important consequences on aquatic invertebrate communities (Dolédec *et al.*, 2006; Kyriakeas & Watzin, 2006; Céréghino *et al.*, 2008; Riens *et al.*, 2013), benthic algal biomass and diatom communities (Urrea & Sabater, 2009; Yu & Lin, 2009; Urrea-Clos *et al.*, 2014) and on leaf decomposition (Hagen *et al.*, 2006; Piscart *et al.*, 2009; Magbanua *et al.*, 2010; Piscart *et al.*, 2011). However, water bodies in agricultural landscapes are potentially exposed to multiple stressors including sediments and pesticides as well and nutrients. Piggott *et al.* (2012) used mesocosms to investigate the individual and combined effects of stressors on benthic invertebrates, algal communities and leaf breakdown. These stressors investigated were raised water temperature, sediment additions and nutrient enrichment. They found that the negative effect of sediments on taxon richness of macroinvertebrates and algae was stronger at higher temperature and that leaf breakdown accelerated with increasing nutrients at ambient temperature. Their results showed that invertebrate community and leaf breakdown can respond to the combined stressors, while algal communities were more influenced than invertebrate communities when stressors acting individually.

To the best of my knowledge, this is the first study investigating impacts of two different agricultural land use (arable and pastoral) on aquatic biodiversity and functioning using two freshwater habitats (ponds and streams), and therefore direct comparing of the study results with previous research was limited, especially for ponds. Further work to investigate the possible effects of other stressors, such as sediments and pesticides, and their interactions on aquatic biodiversity and functioning is required to fully understand the impact of agriculture on the ecology of small water bodies.

In order to understand the causal mechanisms for effects observed, it is also necessary to consider other biotic components including macrophytes, fish and microorganisms, especially those associated with leaf decomposition. It is known that nutrients and macrophytes influence the abundance and diversity of invertebrates (Declerck *et al.*, 2011). Furthermore, changes in macrophyte abundance and diversity caused by eutrophication or herbicide exposure may alter algal community abundance and composition (Bakker *et al.*, 2010). In addition, it has documented that the presence of fish may cause a change in the abundance and/or diversity of invertebrates (Schilling *et al.*, 2009). Ponds and streams used in this study were small, and fish have not been seen during sampling processes. However, fish did occur in some stream sites in the three WFF catchments (Biggs *et al.*, 2014). Macrophyte survey provided by the WFF project showed that pond macrophytes were emergent, floating-leaved and submerged plants whereas in streams only submerged plants were found.

This thesis only investigated the effect of agricultural land use on aquatic macroinvertebrates and algae (diatoms). Future work is to include macrophytes and fish in order to determine the possible effect of the presence of fish and macrophytes on invertebrate and diatom communities. Additionally, using more sites (replicates) in further research would increase the power of the analysis, particularly for streams, which were highly variable among sites. Furthermore, the site-specific land use and water flow direction analyses in this study were done after sampling sites; another aspect should be taken into account when designing future field study in agricultural catchments is to consider these analyses when selecting sites.

7.3. Implications for ecosystem services and sustainable intensification of agriculture

Freshwater ecosystems provide diverse goods and services including food, water, waste treatment and habitats for many organisms (Harrison *et al.*, 2010; Maltby *et al.*, 2011; Suter & Cormier, 2015). In addition, aquatic biodiversity contributes to nutrient cycling and energy flow through nutrient retention, litter decomposition, sediment mixing and water purification (Covich *et al.*, 2004; Maltby *et al.*, 2011; Suter & Cormier, 2015). Furthermore, freshwater ecosystems provide various cultural services including spiritual and religious values of rivers and lakes, and aquatic sports and recreational activities such as fishing, swimming, bathing and canoeing (Harrison *et al.*, 2010; Maltby *et al.*, 2011; Suter & Cormier, 2015). Changes in aquatic biodiversity lead to changes in biological, chemical and physical functions of freshwater (Dudgeon, 2010) that support and help to sustain agricultural production as well as being important for the delivery of other ecosystem services (Cardinale *et al.*, 2012).

Knowledge of the impacts of biodiversity on the functioning of ecosystems has increased rapidly (Loreau *et al.*, 2001; Hooper *et al.*, 2005; Balvanera *et al.*, 2006; Cardinale *et al.*, 2006; Naeem *et al.*, 2009). Recent reviews have collated considerable evidence of the effects of biodiversity loss on ecosystem functioning and on the link between biodiversity and ecosystem services (Cardinale *et al.*, 2012; Balvanera *et al.*, 2014; Harrison *et al.*, 2014; Allan *et al.*, 2015;

Bennett *et al.*, 2015). However, the effect of biodiversity change on ecosystem services is complex and uncertain (Balvanera *et al.*, 2014). For example, Harrison *et al.* (2014) analysed the link between different biodiversity attributes and 11 ecosystem services and reported that five biodiversity attributes (species abundance, species richness, species size/weight, community/habitat area and community/habitat structure) were cited as being important for at least one service in over 50% of papers; three attributes (community/habitat age, aboveground and belowground biomass) were cited as being important for at least one service in 25 to 50% of papers; whereas the functional group attributes were cited as being important for pollination and pest regulation in 14 and 22% of papers.

Changes in biodiversity can be an essential intermediate driver of the impacts of global environmental changes on ecosystem functioning (Isbell *et al.*, 2013) and hence ecosystem services delivery (Cardinale *et al.*, 2012). One service may rely on multiple functions but also one function may contribute to multiple services. Interdependency between services may result in trade-offs or synergies, and understanding the impact of biodiversity change on the interactions and trade-offs among services will depend on those main functions that underpin the functional relations among services (Bennett *et al.*, 2009).

Multiple factors may interact with biodiversity change to affect ecosystem service delivery (Balvanera *et al.*, 2014). For example, a review by Harrison *et al.* (2014) 22% of papers showed a link between abiotic factors that interacting with biodiversity and ecosystem service delivery. Furthermore, the effect of biodiversity on ecosystem service delivery could be positive or negative (Harrison *et al.*, 2014) and it could be changed over time (Isbell *et al.*, 2013). For example, in a long-term grassland field experiment, Isbell *et al.* (2013) found that nitrogen enrichment increased plant productivity; however, this effect declined over time, and it also reduced the number of plant species.

Understanding how agricultural practices impact on ecosystem service provision is important for effective risk assessment and environmental management (Maltby, 2013). In the current study, the findings relating to the effects of land use on aquatic macroinvertebrates and diatoms provide valuable information for biodiversity changes (especially community composition) and how these changes may affect the ecosystem functioning and associated ecosystem services. For example, changes in algal community structure may influence nutrient cycling and hence improve water quality. Cardinale (2011) demonstrated that nitrogen uptake rates by benthic algae increased with increasing species richness. As diatom species richness was lower in arable ponds than pastoral ponds the ability of algae to remove nitrogen and improve water quality may be lower in arable ponds. In addition, several invertebrate species have high aesthetic value and Odonata (dragonflies and damselflies) were more abundant in arable ponds than in pastoral ponds. It has been demonstrated that number of odonate species increased with increasing macrophyte diversity (Carchini *et al.*, 2007) and decreased with elevated concentrations of phosphorus and ammonia (Carchini *et al.*, 2005). In addition, using ponds for livestock watering will damage the marginal vegetation which may result in high turbidity and decreased macrophyte coverage of ponds (Carchini *et al.*, 2005).

Agricultural ecosystems must be managed for multiple ecosystem services including food production, pest regulation and soil maintenance and productivity (Foresight, 2011; Balvanera *et al.*, 2014). There is increasing interest in using sustainable intensification (Godfray *et al.*, 2010) that is increasing food production in the same area of land with reducing impacts on the environment (Godfray *et al.*, 2010; Foley *et al.*, 2011; Godfray & Garnett, 2014). Foley *et al.* (2011) analysed ways to achieve this sustainability, including stopping agricultural expansion, increasing farming efficiency, reducing food waste and shifting diets. They concluded that these solutions could double food production and reduce the impacts of agriculture on environment. Godfray and Garnett (2014) concluded that increasing food production on the land area should ensure food security and converting more land to agriculture could cause significant damage to the environment. Sustainable intensification requires understanding environmental impacts and working with nature to maintain or enhance essential ecosystem services to ensure future human well-being.

The results of this thesis suggest that knowing the effects of agricultural land use on freshwater communities can be important to understanding their long term effects on the ecology and ecosystem services and the implications for sustainable food production. New strategies of land use management are needed to reduce the impacts of agricultural intensification on freshwater ecosystem services and maintain aquatic biodiversity (Foley *et al.*, 2011; Godfray & Garnett, 2014). Such managements and technologies increase food production and do not harm the environment.

7.4. Conclusion

Agricultural land use can adversely affect the structure and functioning of aquatic communities. Both ponds and streams are affected by arable and pastoral land use but they differ substantially in their responses. Algal biomass was higher, but macroinvertebrate abundance and taxa richness was lower, in arable streams. Algal biomass and leaf litter processing were also higher in arable ponds, but diatom diversity was lower. Given that, it is clear that arable land use had prominent effects on stream condition, but pastoral land use had prominent effects on pond condition. This study suggests that pastoral land use that is associated with higher phosphorus levels has greater impact on pond diatoms while arable land use that is potentially associated with high levels of nutrients (mainly nitrogen) and sediments has greater impact on macroinvertebrates in streams. This study also suggests that intraspecific interactions can affect ecosystem functioning (i.e. leaf litter decomposition) and this effect will be stronger with increasing temperature. Changes in agricultural land use may have considerable potential impact on the ecology and ecosystem services provided by freshwater habitats.

In order to protect freshwater ecosystems, understanding the possible effects of agricultural land use on community structure and functioning of freshwater ecosystems is extremely important. Increase in understanding should help in identify the best land use management to maintain sustainable agricultural production and protect freshwater habitats and the ecosystem services they provide.

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Appendix A

Table A1: Number of macroinvertebrate taxa found in each **pond**; AP = arable pond and PP = pastoral pond.

Taxa	**Family**	**AP1**	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**PP1**	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**
Tricladida	Planariidae *Polycelis*	6	602	7	2	0	110	120	0	5	0	0	0
Gastropoda	Bithyniidae	0	0	9	0	0	0	0	0	0	0	0	0
Gastropoda	Hydrobiidae	0	0	0	24	0	2	1	0	1	1	0	0
Gastropoda	Lymnaeidae	14	0	15	93	5	47	0	0	0	52	16	0
Gastropoda	Planorbidae	1	1	121	20	0	31	166	0	0	287	7	1256
Bivalvia	Sphaeriidae	4	0	56	5	1	6	18	66	0	70	0	0
Rhynchobdella	Glossiphoniidae	9	7	1	0	11	26	5	2	0	0	0	0
Arhynchobdella	Erpobdellidae	4	0	0	0	0	2	0	0	0	9	9	10
Oligochaeta		24	82	17	5	14	149	33	211	296	12	11	47
Acari	Hydrachnida	16	2	31	10	0	16	278	0	0	4	0	16
Isopoda	Asellidae	9	1	28	0	0	0	30	0	0	50	21	0
Amphipoda	Gammaridae	1	0	64	107	0	73	0	0	1	12	0	0
Amphipoda	Crangonyctidae	137	0	76	0	0	0	0	0	0	0	0	0
Ephemeroptera	Baetidae	3	0	67	89	0	12	390	0	30	0	1	1
Ephemeroptera	Caenidae	0	0	115	74	0	0	0	0	0	0	0	0
Ephemeroptera	Leptophlebiidae	0	0	0	0	0	0	0	0	9	1	0	0
Plecoptera	Nemouridae	0	5	0	0	0	0	3	0	0	0	0	0
Odonata	Coenagrionidae	22	0	97	120	0	5	31	0	0	0	0	0
Odonata	Aeshnidae	2	0	1	0	0	7	0	0	0	0	0	0
Odonata	Libellulidae	0	0	2	0	0	0	3	0	0	0	0	0
Hemiptera	Corixidae	13	0	110	344	0	81	35	0	3	0	1	4
Hemiptera	Gerridae	0	0	2	15	0	0	5	0	0	0	0	0
Hemiptera	Hydrometridae	0	0	0	0	0	0	1	0	0	0	0	0
Hemiptera	Naucoridae	0	0	0	1	0	0	25	0	0	0	0	0
Hemiptera	Notonectidae	0	0	0	0	0	13	3	0	0	0	0	0
Hemiptera	Pleidae	0	0	4	0	0	0	70	0	0	0	0	0
Hemiptera	Veliidae	0	0	2	0	0	12	0	0	0	0	0	0

Table A1: Continued.

Taxa	Family	AP1	AP2	AP3	AP4	AP5	AP6	PP1	PP2	PP3	PP4	PP5	PP6
Coleoptera	Curculionidae	9	4	0	0	0	1	10	0	2	1	0	8
Coleoptera	Dytiscidae	57	139	2	1	35	95	258	86	153	1	241	63
Coleoptera	Elmidae	0	0	0	2	0	0	0	0	0	0	0	0
Coleoptera	Haliplidae	2	0	13	0	0	12	64	0	2	0	1	0
Coleoptera	Helophoridae	25	11	22	4	1	1	107	15	57	13	140	76
Coleoptera	Hydraenidae	0	0	0	0	0	0	0	0	2	0	0	0
Coleoptera	Hydrophilidae	9	40	5	7	5	24	82	5	82	13	3	40
Coleoptera	Noteridae	1	0	3	0	0	0	18	0	0	0	0	0
Coleoptera	Scirtidae Larvae	0	2	1	10	23	2	427	0	6	6	0	0
Trichoptera	Beraeidae	0	4	0	0	0	0	0	0	0	0	0	0
Trichoptera	Glossosomatidae	0	0	0	0	0	0	0	0	1	0	0	0
Trichoptera	Hydroptilidae	0	0	7	0	0	0	0	0	0	0	0	0
Trichoptera	Hydropsychidae	0	0	0	0	0	0	0	0	1	0	0	0
Trichoptera	Leptoceridae	1	0	8	25	0	0	70	0	0	0	0	0
Trichoptera	Limnephilidae	10	5	4	59	5	27	48	39	0	0	1	0
Trichoptera	Molannidae	0	0	0	2	0	0	0	0	0	0	0	0
Trichoptera	Polycentropodidae	0	0	0	0	0	1	0	0	0	0	0	0
Trichoptera	Psychomyiidae	0	0	0	0	0	2	0	0	0	0	0	0
Diptera	Ceratopogonidae	1	2	72	30	24	53	48	0	257	6	2	2
Diptera	Chaoboridae	4	0	0	0	0	96	119	0	0	0	0	0
Diptera	Chironomidae	88	622	5063	1742	347	2013	2053	9	683	23	107	129
Diptera	Culicidae	7	18	3	0	224	15	3	1	17	5	0	2
Diptera	Dixidae	0	0	14	7	0	0	0	0	0	0	0	0
Diptera	Empididae	0	0	9	14	0	0	0	0	0	0	0	0
Diptera	Limoniidae	0	1	0	0	1	0	2	1	0	0	0	0
Diptera	Psychodidae	0	65	0	0	0	7	6	10	0	6	4	1
Diptera	Ptychopteridae	0	0	0	0	0	2	5	0	1	0	0	0
Diptera	Stratiomyidae	0	0	1	0	0	0	91	0	1	0	0	4
Diptera	Syrphidae	0	0	0	0	21	0	1	0	26	2	0	7

Table A1: Continued.

Taxa	**Family**	**AP1**	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**PP1**	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**
Diptera	Tabanidae	0	0	0	0	0	0	1	0	0	0	0	0
Diptera	Tipulidae	0	8	4	4	0	0	2	0	1	7	0	2
Lepidoptera		1	4	8	0	2	0	12	0	3	0	3	0

Table A2: Number of macroinvertebrate taxa found in each **stream**; AS = arable stream and PS = pastoral stream.

Taxa	**Family**	**AS1**	**AS2**	**AS3**	**AS4**	**AS5**	**AS6**	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**
Tricladida	Planariidae *Polycelis*	0	1	0	603	19	0	95	4	12	118	41	6
Gastropoda	Hydrobiidae	2	864	0	3	0	12	70	636	356	4483	231	0
Gastropoda	Lymnaeidae	0	0	0	0	0	0	0	7	0	1	0	0
Bivalvia	Sphaeriidae	42	291	86	1	27	7	284	1283	41	6	27	8
Rhynchobdella	Glossiphoniidae	0	0	0	23	2	1	0	0	1	2	3	1
Arhynchobdella	Erpobdellidae	0	0	0	3	7	0	0	1	0	0	0	0
Oligochaeta		11	4	40	891	175	56	35	4	5	0	7	0
Acari	Hydrachnida	0	0	0	6	0	0	2	1	6	2	0	1
Isopoda	Asellidae	0	0	0	1	0	0	0	0	0	5	0	4
Amphipoda	Gammaridae	1069	271	162	1	129	999	299	2101	1042	439	1192	2077
Ephemeroptera	Baetidae	0	143	233	0	15	28	0	1	147	35	2	1
Ephemeroptera	Ephemeridae	0	2	0	0	0	0	0	0	0	0	1	0
Ephemeroptera	Leptophlebiidae	1	12	45	0	25	0	0	1	0	18	6	0
Plecoptera	Nemouridae	2	0	8	0	0	0	186	185	36	167	0	6
Odonata	Coenagrionidae	0	0	0	0	0	0	0	0	0	0	0	1
Odonata	Aeshnidae	0	0	0	0	0	0	0	0	0	0	1	0
Hemiptera	Nepidae	0	0	0	0	0	0	0	0	0	0	1	0
Hemiptera	Veliidae	1	0	15	5	12	9	14	2	0	0	21	5
Coleoptera	Curculionidae	4	0	0	0	1	0	5	1	0	0	1	0
Coleoptera	Dytiscidae	1	0	1	0	0	0	0	0	0	3	0	0
Coleoptera	Elmidae	0	6	0	6	15	1	0	8	1	4	76	1
Coleoptera	Helophoridae	0	0	13	2	1	1	0	0	0	1	10	0
Coleoptera	Hydraenidae	1	0	1	0	0	1	0	18	0	0	0	0
Coleoptera	Hydrophilidae	0	0	2	1	0	0	0	0	0	4	3	0
Coleoptera	Scirtidae Larvae	8	0	49	1	9	79	30	359	43	131	74	20
Trichoptera	Beraeidae	0	0	0	0	0	0	0	5	0	0	0	0
Trichoptera	Glossosomatidae	0	1	0	0	10	5	0	0	26	0	9	2
Trichoptera	Goeridae	0	0	0	0	0	0	0	0	0	0	1	0
Trichoptera	Lepidostomatidae	0	0	0	0	0	0	0	0	9	0	0	0

Table A2: Continued.

Taxa	Family	AS1	AS2	AS3	AS4	AS5	AS6	PS1	PS2	PS3	PS4	PS5	PS6
Trichoptera	Leptoceridae	0	0	0	0	1	0	0	0	0	0	0	0
Trichoptera	Limnephilidae	58	10	63	2	45	29	14	84	12	310	30	5
Trichoptera	Polycentropodidae	1	0	2	0	0	9	19	59	15	0	1	4
Trichoptera	Psychomyiidae	0	0	0	0	0	4	0	2	5	0	0	0
Megaloptera	Sialidae	0	0	2	0	0	0	0	0	0	0	0	0
Diptera	Ceratopogonidae	17	0	4	319	34	16	1	22	1	33	17	16
Diptera	Chaoboridae	0	0	0	0	0	0	0	0	0	0	0	2
Diptera	Chironomidae	233	38	276	456	319	95	250	221	147	1169	189	680
Diptera	Dixidae	0	0	0	1	0	3	4	14	4	7	2	6
Diptera	Empididae	0	0	0	0	0	0	1	0	1	2	0	0
Diptera	Limoniidae	0	21	1	0	1	0	6	8	17	1	7	6
Diptera	Pedicidae	5	2	2	0	2	12	4	5	1	0	0	8
Diptera	Psychodidae	1	2	1	5	6	0	1	21	6	29	0	5
Diptera	Ptychopteridae	2	0	2	12	2	49	5	107	1	0	24	50
Diptera	Simuliidae	1	9	4	0	1	0	0	15	5	2367	0	0
Diptera	Stratiomyidae	0	0	0	0	0	0	0	2	1	2	2	5
Diptera	Tipulidae	0	2	0	0	1	0	1	0	1	1	0	0
Lepidoptera		0	0	1	1	2	1	0	0	0	2	0	0

Appendix B

Table B1: Relative abundance of diatom species found in each **pond**; AP = arable pond and PP = pastoral pond; (absent = –, 0.1-1% = *, 1-10%= **, 10-30% = ***, 30-50% = ****, ≥ 50% = *****)

Diatom Species	**Abbreviation**	**AP1**	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**PP1**	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**
Achnanthes oblongella	A.obl	–	–	–	–	–	–	–	–	–	*	–	–
Achnanthidium minutissimum	A.min	*****	***	*****	*****	***	*****	*****	***	**	***	***	***
Amphora libyca	A.lib	–	–	*	–	*	*	–	*	**	*	*	–
Amphora pediculus	A.ped	–	–	*	*	–	–	*	*	**	*	*	–
Caloneis bacillum	C.bac	–	–	–	–	–	–	*	*	–	*	–	–
Cocconeis placentula	C.pla	–	*	*	*	–	*	*	*	*	**	–	–
Craticula ambigua	C.amb	–	–	–	–	–	–	–	–	–	–	*	*
Cyclotella meneghiniana	C.men	–	–	*	–	–	–	–	–	–	–	–	–
Cyclotella sp.	C.sp.	***	–	–	–	–	–	–	–	–	–	–	–
Cymatopleura solea	C.sol	–	–	–	–	*	–	–	–	–	–	–	–
Cymbella cistula	C.cis	–	–	*	*	–	–	–	*	–	–	–	–
Cymbella cuspidata	C.cus	–	–	–	–	–	–	–	*	–	–	–	–
Cymbella lanceolata	C.lan	–	–	–	–	*	–	–	–	–	–	–	**
Cymbella microcephala	C.mic	–	–	*	**	–	–	–	–	–	–	–	–
Diadesmis contenta	D.con	–	–	–	–	–	–	–	–	–	–	*	–
Diatoma mesodon	D.mes	–	–	*	*	–	–	–	–	–	–	–	–
Diatoma tenue	D.ten	–	–	*	–	–	–	–	–	–	–	–	–
Diploneis elliptica	D.ell	–	–	–	–	–	–	–	*	–	–	–	–
Diploneis oblongella	D.obl	–	–	–	*	*	**	–	**	–	–	–	–
Encyonema silesiacum	E.sil	*	–	*	**	*	*	*	–	–	*	–	**
Eunotia bilunaris	E.bil	**	*****	–	–	*	*	–	*	***	**	*	**
Eunotia exigua	E.exi	*	–	–	–	–	–	*	*	–	–	*	*
Eunotia minor	E.min	–	**	*	–	–	–	–	–	–	–	–	–
Fragilaria capucina	F.cap	*	–	*	**	****	**	**	*	–	**	–	**
Fragilaria tenera	F.ten	–	–	–	–	–	*	–	*	–	–	*	–
Fragilaria vaucheriae	F.vau	–	–	–	–	**	*	–	–	–	–	**	–

Table B1: Continued

Diatom Species	**Abbreviation**	**AP1**	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**PP1**	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**
Frustulia vulgaris	F.vul	*	*	–	–	–	–	–	*	–	*	–	–
Gomphonema acuminatum	G.acu	**	–	–	–	–	–	*	–	–	–	–	–
Gomphonema angustatum	G.ang	*	**	–	*	**	*	*	**	*	**	**	**
Gomphonema clavatum	G.cla	–	–	–	–	*	*	*	*	*	*	**	–
Gomphonema gracile	G.gra	–	–	–	–	–	–	*	–	–	*	–	–
Gomphonema olivaceum	G.oli	–	–	–	–	–	–	–	*	–	–	–	–
Gomphonema parvulum	G.par	*	**	–	–	*	–	**	–	*	*	**	**
Gomphonema truncatum	G.tru	–	–	–	*	**	–	–	**	–	–	*	–
Gyrosigma acuminatum	Gy.acu	–	–	*	–	–	*	–	*	–	–	–	–
Hantzschia amphioxys	H.amp	–	*	–	–	–	–	*	*	–	*	*	–
Lemnicola hungarica	L.hun	–	–	–	–	–	–	–	–	–	*	–	–
Luticola ventricosa	L.ven	*	–	–	–	–	–	–	*	–	–	*	–
Melosira varians	M.var	–	–	*	–	**	–	–	–	–	–	–	–
Meridion circulare	M.cir	*	–	*	–	*	*	–	*	–	*	–	–
Navicula atomus	N.ato	–	–	–	–	**	–	–	–	–	–	*	–
Navicula capitata	N.cap	–	–	–	*	–	–	–	–	–	–	–	–
Navicula capitatoradiata	N.capd	*	*	–	–	–	–	–	–	–	*	*	–
Navicula cari	N.car	–	–	–	–	–	–	–	**	–	–	*	–
Navicula cryptocephala	N.cryc	–	–	–	–	*	*	–	–	–	–	–	***
Navicula cryptonella	N.cryn	*	–	*	*	**	–	–	–	*	*	–	*
Navicula gregaria	N.gre	**	*	*	*	–	*	**	***	**	***	**	*
Navicula lanceolata	N.lan	*	*	*	–	**	**	**	**	**	*	–	–
Navicula minima	N.min	*	*	*	–	*	–	**	**	**	**	*	**
Navicula menisculus	N.men	*	–	–	–	–	–	–	–	–	–	–	–
Navicula molestiformis	N.mol	–	–	–	–	–	–	*	–	–	–	–	–
Navicula radiosa	N.rad	–	–	*	–	**	–	–	**	–	–	*	*
Navicula slesvicensis	N.sle	–	–	–	–	–	–	–	–	–	*	–	–
Navicula tripunctata	N.trip	*	–	–	*	–	–	–	*	*	*	–	–
Navicula trivialis	N.tri	–	–	–	–	–	–	–	–	–	–	**	–

Table B1: Continued

Diatom Species	**Abbreviation**	**AP1**	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**PP1**	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**
Navicula veneta	N.ven	–	–	–	–	–	–	–	–	–	***	**	–
Nitzschia acicularis	N.aci	*	–	–	–	–	–	–	–	–	–	–	–
Nitzschia amphibia	N.amp	–	–	–	–	–	–	–	**	–	–	**	*
Nitzschia capitellata	N.cap	–	–	–	–	–	–	–	–	–	*	–	–
Nitzschia dissipata	N.dis	**	–	*	**	*	–	–	–	–	*	–	–
Nitzschia linearis	N.lin	–	–	–	–	*	*	–	–	*	*	*	–
Nitzschia palea	N.pal	**	**	*	*	*	*	*	*	**	***	**	**
Nitzschia pusilla	N.pus	*	*	–	–	–	*	*	–	*	*	*	–
Nitzschia sigma	N.sig	–	–	–	–	–	*	–	–	–	–	–	–
Nitzschia sp.	N.sp.	–	–	–	–	–	–	–	–	–	–	–	****
Pinnularia appendiculata	P.app	*	*	–	–	–	–	–	**	–	*	–	–
Pinnularia subcapitata	P.sub	–	*	–	–	–	–	–	–	–	*	–	–
Pinnularia viridis	P.vir	–	*	–	–	–	–	–	–	–	*	*	–
Placoneis clementis	P.cle	–	–	–	*	–	–	–	–	–	–	–	–
Placoneis elginensis	P.elg	–	–	–	–	–	–	–	*	*	*	–	–
Planothidium delicatulum	P.del	–	–	–	–	–	–	–	*	–	–	–	–
Planothidium frequentissimum	P.fre	*	*	*	–	*	*	**	***	***	***	***	**
Planothidium lanceolata	P.lan	*	–	*	–	*	*	**	**	****	**	**	*
Reimeria sinuata	R.sin	–	–	–	–	–	*	–	–	–	–	–	–
Sellaphora seminulum	S.sem	–	–	–	–	*	–	–	–	–	–	–	–
Sellaphora pupula	S.pup	–	–	–	–	–	–	–	**	**	**	**	–
Stauroneis anceps	S.anc	*	–	–	–	*	*	–	*	**	*	*	–
Stauroneis kriegeri	S.kri	*	–	–	–	–	–	–	–	–	*	–	–
Stauroneis phoenicenteron	S.pho	–	*	–	–	*	–	–	*	*	**	–	–
Stauroneis smithii	S.smi	–	–	–	–	–	–	–	**	*	–	*	–
Surirella angusta	S.ang	*	*	–	–	–	*	*	–	*	–	*	–
Surirella brebisonii	S.bre	**	–	–	–	–	–	–	*	*	*	*	–
Surirella minuta	S.min	–	–	–	–	–	–	–	*	–	–	–	–
Synedra acus	S.acu	*	–	–	–	***	**	–	*	*	*	*	–

Table B1: Continued

Diatom Species	**Abbreviation**	**AP1**	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**PP1**	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**
Synedra parasitica	S.par	**–**	**–**	**–**	*****	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**
Tryblionella apiculata	T.api	**–**	**–**	**–**	**–**	**–**	*****	**–**	**–**	*****	**–**	**–**	**–**
Tryblionella hungarica	T.hun	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	******	**–**

Table B2: Relative abundance of diatom species found in each **stream**; AS = arable stream and PS = pastoral stream; (absent = –, 0.1-1% = *, 1-10% = **, 10-30% = ***, 30-50% = ****, ≥ 50% = *****)

Diatom Species	**Abbreviation**	**AS1**	**AS2**	**AS3**	**AS4**	**AS5**	**AS6**	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**
Achnanthidium minutissimum	A.min	*****	****	****	**	***	****	*****	****	*****	*****	*****	****
Amphora libyca	A.lib	–	–	–	–	–	–	–	–	–	–	–	*
Amphora pediculus	A.ped	**	*	*	*	**	**	**	*	**	**	**	***
Caloneis bacillum	C.bac	–	–	–	–	–	*	–	–	–	–	–	–
Cocconeis placentula	C.pla	**	***	–	–	*	–	**	*	–	*	**	*
Diatoma mesodon	D.mes	*	–	–	–	–	–	–	–	–	–	–	–
Diploneis elliptica	D.ell	–	–	–	–	–	–	–	–	–	–	–	*
Diploneis oblongella	D.obl	*	–	–	–	–	–	*	*	–	–	–	*
Encyonema minutum	E.min	–	*	–	–	–	–	–	–	–	–	–	–
Encyonema silesiacum	E.sil	–	–	–	–	*	–	–	–	–	–	–	–
Fragilaria capucina	F.cap	–	–	–	–	–	–	–	*	–	–	–	–
Fragilaria vaucheriae	F.vau	–	–	–	–	–	–	–	–	–	–	–	*
Frustulia vulgaris	F.vul	–	–	*	–	–	*	*	*	–	–	–	–
Gomphonema angustatum	G.ang	**	–	**	*	*	**	**	*	**	*	**	**
Gomphonema gracile	G.gra	–	–	–	–	–	–	–	–	–	–	–	*
Gomphonema olivaceum	G.oli	–	–	–	*	–	–	–	*	–	**	*	–
Gomphonema parvulum	G.par	–	*	–	–	–	–	–	–	*	–	*	*
Hantzschia amphioxys	H.amp	*	–	*	–	–	–	–	*	–	–	–	–
Meridion circulare	M.cir	***	*	**	*	*	**	**	**	–	*	*	***
Navicula atomus	N.ato	–	–	–	*	–	–	–	–	–	–	–	–
Navicula capitata	N.cap	–	–	–	–	*	–	–	–	–	–	–	–
Navicula cryptocephala	N.cryc	–	–	–	–	–	–	*	–	–	–	–	–
Navicula cryptotenella	N.cryn	–	–	–	–	**	**	–	*	–	–	**	–
Navicula gregaria	N.gre	**	**	**	****	**	*	**	**	–	*	*	*
Navicula lanceolata	N.lan	*	**	***	****	*****	*	*	****	*	**	**	–
Navicula minima	N.min	**	*	–	*	*	–	**	–	*	–	*	*
Navicula menisculus	N.men	*	*	–	–	–	–	*	**	*	–	–	–

Table B2: Continued

Diatom Species	**Abbreviation**	**AS1**	**AS2**	**AS3**	**AS4**	**AS5**	**AS6**	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**
Navicula molestiformis	N.mol	*	**	**	*	*	*	–	**	–	–	*	–
Navicula tripunctata	N.trip	**	**	*	–	*	**	*	**	**	*	**	*
Nitzschia amphibia	N.amp	–	*	–	*	–	–	–	–	–	–	*	–
Nitzschia dissipata	N.dis	**	*	**	**	**	****	*	*	–	–	**	*
Nitzschia linearis	N.lin	–	–	**	*	*	–	–	–	–	–	*	*
Nitzschia palea	N.pal	–	–	*	**	**	–	–	–	–	*	**	–
Planothidium frequentissimum	P.fre	**	**	*	**	*	*	**	*	**	–	*	**
Planothidium lanceolata	P.lan	**	***	**	**	**	**	**	*	*	*	**	***
Pseudostaurosira brevistriata	P.bre	–	*	–	–	–	–	–	–	–	–	–	–
Reimeria sinuata	R.sin	–	**	–	–	*	–	–	–	*	*	–	–
Rhoicosphenia abbreviata	R.abb	*	–	–	–	–	*	*	–	*	–	**	*
Sellaphora pupula	S.pup	–	–	–	–	–	–	–	–	–	–	–	*
Stauroneis anceps	S.anc	–	–	*	–	–	–	–	–	–	–	–	–
Stauroneis smithii	S.smi	–	–	–	–	–	–	*	–	–	–	–	–
Surirella angusta	S.ang	–	–	*	–	*	–	–	–	–	–	–	*
Surirella brebisonii	S.bre	**	*	***	**	**	*	**	**	–	*	**	**
Synedra ulna	S.uln	–	–	–	–	–	–	–	–	–	*	–	–
Tryblionella hungarica	T.hun	–	*	–	–	–	–	–	–	–	–	–	–