The role of detrital subsidies for biological control by generalist predators evaluated by molecular gut content analysis

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Only two things are infinite,
the universe and human stupidity,
and I'm not sure about the former.
Albert Einstein

Erschrecke die Wirklichkeit zu Tode:
zeige ihr, dass sie nur Eine von vielen
Möglichkeiten ist.
Werner T. Küstenmacher

PEER-REVIEWED PUBLICATIONS IN THIS THESIS

von Berg K, Thies C, Tscharntke T, Scheu S (in prep.) Detrital subsidies increase herbivore control in cereal fields by fostering the impact of individual predator species.

von Berg K, Thies C, Tscharntke T, Scheu S (resubmitted) Cereal aphid control by generalist predators in presence of belowground alternative prey: complementary predation as affected by prey density. *Oecologia.*

von Berg K, Traugott M, Symondson WOC, Scheu S (submitted) Impact of abiotic factors on predator-prey interactions: DNA-based gut content analysis in a microcosm experiment. *Bulletin of Entomological Research*.

von Berg K, Traugott M, Micic A, Symondson WOC, Scheu S (in prep.) DNA-based identification of scavenging and predation in a guild of generalist predators: a mesocosm study employing multiplex PCR.

von Berg K, Traugott M, Symondson WOC, Scheu S (submitted) The effects of temperature on detection of prey DNA in two species of carabid beetles. *Bulletin of Entomological Research*.

FURTHER PEER-REVIEWED PUBLICATIONS

Birkhofer K, Gavish-Regev E, Endlweber K, Lubin YD, von Berg K, Wise DH, Scheu S (submitted) Cursorial spiders retard initial aphid population growth at low densities in winter wheat: results of a cage experiment and DNA-based gut content analysis. *Bulletin of Entomological Research*.

Zusammenfassung

Generalistische Prädatoren können wichtige Ökosystemdienste zur Verfügung stellen indem sie Pflanzenfresser-Populationen eindämmen. Durch effektive biologische Schädlingskontrolle kann die Ernte gesteigert und der Einsatz von Pestiziden reduziert werden, was beides ökonomische Vorteile für Landwirte darstellt. Die vorliegende Arbeit untersuchte den Einfluss von generalistischen Prädatoren auf Blattlauspopulationen im Winterweizen, Faktoren die diesen Einfluss verändern und Werkzeuge die helfen, Räuber-Beute Beziehungen besser zu verstehen.

Die Verbesserung der Habitate sowohl in Feldern als auch in deren Umgebung ist ein Hauptanliegen natürlicher biologischen Schädlingskontrolle. Daher konzentrierte sich das erste Feldexperiment auf den Einfluss einer organischen Feldzugabe (Maismulch) auf die Schädlingsbekämpfung durch das Erhöhen von alternativer Beute aus dem Zersetzersystem. Kohlenstoff aus dem Maismulch, welcher kurz vor der Aussaat des Winterweizens auf die Felder ausgebracht wurde, wurde sowohl von im Boden lebenden Zersetzern als auch von Räubern inkorporiert, was auf vielfältige trophische Verbindungen im unterirdischen System schließen lässt. Maisgebürtiger Kohlenstoff wurde ebenfalls in zwei oberirdisch lebenden generalistischen Prädatoren nachgewiesen, einem Laufkäfer und einem Kurzflügelkäfer. Die Dichten dieser zwei Arten sowie die einiger anderer generalistischer Prädatoren waren durch die Zugabe von Maismulch signifikant erhöht. Passend hierzu waren die Blattlausdichten ausschließlich in den gemulchten Feldern signifikant erniedrigt. Die Ergebnisse lassen Verbindungen des oberirdischen und des unterirdischen Systems durch generalistische Prädatoren vermuten, was die Applikation von organischem Substrat zu einem wirkungsvollem Werkzeug macht um biologische Schädlingskontrolle zu fördern.

Das Vorhandensein von alternativer Beute kann eventuell generalistische Prädatoren von der zu bekämpfenden Beute ablenken; daher konzentrierte sich das zweite Feldexperiment auf den gleichzeitigen Effekt von generalistischen Prädatoren auf Blattlauspopulationen und auf vorhandene alternative Beute. Generalistische Prädatoren erniedrigten Blattlauspopulationen

unter die ökonomisch relevante Schadschwelle, und das sogar bei hohen Initial-Blattlausdichten. Collembolendichten wurden ebenfalls, ungeachtet der Blattlausdichte, von generalistischen Prädatoren erniedrigt, was auf eine sich ergänzende Ernährung von beiden Beutearten schließen lässt. Darüber hinaus wechselten generalistische Prädatoren von unterirdischer Beute (Schnellkäfer) zu Blattläusen wenn diese an Dichte zunahmen. Die Ergebnisse zeigten dass alternative Beute die Kontrolle von Blattlauspopulationen nicht stört. Dies unterstützt die Idee, biologische Schädlingskontrolle durch das Erhöhen von alternativer Beute zu stärken.

Abiotische Faktoren können Räuber-Beute Beziehungen verändern und dadurch biologische Schädlingskontrolle beeinflussen. Der Effekt von Regen auf den Einfluss von generalistischen Prädatoren auf Blattläuse wurde in einem Mikrokosmos-Experiment untersucht. Regen entfernte Blattläuse von den Weizenpflanzen, was die Verfügbarkeit von Blattlausbeute auf dem Boden erhöhte und zu einem erhöhten Blattlausfraß durch generalistische Prädatoren führte. Dies änderte jedoch nicht den Blattlausbefall was vermuten lässt, dass die Räuber bevorzugt an toten Läusen gefressen haben. Fraß an toter Beute wurde in einem weiteren Mesokosmos-Experiment untersucht; alle untersuchten Arten der generalistischen Prädatoren außer einer Wolfsspinne fraßen tote Läuse was beweist dass das Konsumieren von toter Beute weit verbreitet bei Lauf- und Kurzflügelkäfern ist.

Für die Analyse der Mikro- und Mesokosmos-Experimente wurde eine DNA-basierte Darminhaltsanalyse verwendet die es ermöglichte, trophische Verbindungen genauestens zu untersuchen. Um die Anwendbarkeit dieser Technik weiter zu verbessern wurden Fütterungsversuche im Labor durchgeführt um Faktoren zu untersuchen, die die Nachweisbarkeit von Beute-DNA im Darm beeinflussen. Umgebungstemperaturen unter 20 °C hatten keine Auswirkungen auf die Nachweisbarkeit von Beute-DNA, daher dürfte die Temperatur nur eine untergeordnete Rolle spielen im Bezug auf DNA Darminhaltsanalysen. Getestet wurden auch die Nachweisbarkeitsraten von vier unterschiedlich langen Fragmenten, wobei das Längste Fragment die Nachweisbarkeit deutlich verkürzte und das Kürzeste Fragment am Längsten nachweisbar war. Daher ist es denkbar Primerpaare, die Fragmente von deutlich unterschiedlicher Länge amplifizieren, nicht nur im Nachweis von Prädationsraten zu verwenden sondern auch um den Zeitpunkt zu bestimmen, wann die Beute

gefressen wurde. Jedoch ist zu bedenken, dass die Nachweisbarkeit von Beute DNA sogar in sehr eng verwandten Räuberarten unterschiedlich sein kann.

Die vorliegende Arbeit zeigt dass biologische Schädlingskontrolle durch die Applikation von organischem Material gefördert werden kann, aber die Ergebnisse lassen vermuten dass für die Entwicklung von effizienten Kontrollstrategien abiotische Faktoren sowie Habitat Eigenheiten beachtet werden müssen. DNA basierte Darminhaltsanalysen von Räubern zeigten sich als sehr hilfreich in der Untersuchung von Räuber-Beute Beziehungen in vereinfachten Untersuchungssystemen. Umgebungstemperatur, amplifizierte Fragmentlänge und Artidentität des Räubers zeigten Effekte auf die Nachweisbarkeit von Beute-DNA im Darm von Räubern. Diese Faktoren als auch der Konsum von toter Beute müssen in der Evaluierung von Prädationsraten im Freiland und damit von Effekten im Sinne biologischer Schädlingskontrolle durch generalistische Prädatoren berücksichtigt werden.

Summary

Generalist predators can provide important ecosystem services by reducing herbivore populations in agricultural systems. Effective biological control can increase plant yield and reduce the need of pesticide application, both being profitable for farmers. The present work investigated the impact of generalist predators on aphid populations in winter wheat, factors modulating these effects and tools helping to better understand predator-prey interactions.

Improving habitats within and aside arable fields for generalist predators is of major concern in conservation biological control, therefore the first field experiment focused on the impact of a detrital subsidy on herbivore suppression via increasing alternative prey from the microbe-detritivore subsystem. The maize mulch, applied shortly before winter wheat was sown, was incorporated by belowground decomposers as well as predators, indicating diverse trophic links in the belowground subsystem. Two aboveground generalist predators, one carabid and one staphylinid, also had incorporated maize-born carbon. These predators along with several other species were significantly increased in mulched fields. Consequently, only in mulched fields aphid populations were significantly decreased. The results suggest linkages between the belowground and aboveground system via generalist predators, showing detrital subsidies to be a valuable tool to foster biological control. As these effects were not consistent between fields, additional factors as well as the temporal and spatial scale have to be considered in evaluating biological control effects.

The presence of alternative prey might distract generalist predators from the target prey; therefore the second field experiment focused on the effect of generalist predators on aphid populations as well as on abundant alternative prey affected by different aphid densities. Generalist predators decreased aphid densities even in the high density treatment below the threshold of economical damage. Collembolans were also decreased by generalist predators in all three aphid density treatments, indicating complementary predation of these two prey species. Moreover, generalist predators shifted from abundant belowground prey (click beetles) to aphid prey at increasing aphid densities. The results demonstrated non-disruptive

effects of alternative prey on aphid populations, supporting the idea of alternative prey enhancement for improving biological control.

Abiotic factors can modulate predator-prey interactions, thereby modifying biological control effects. In a microcosm experiment the effect of rain on aphid predation by ground dwelling predators was analysed. Rain significantly dislodged aphids from wheat plants, increasing aphid prey availability on the soil surface. In fact, more ground dwelling predators had consumed aphids in the rain treatments compared to the no rain treatments. However, synergistic effects on aphid populations were absent, suggesting a preference for dead aphid prey by ground dwelling predators. Feeding on dead prey (scavenging) is supposed to be common in generalist predators, and assessing scavenging and active predation was addressed in a mesocosm experiment. Scavenging was found in all investigated generalist predators except a lycosid spider, indicating the commonness of scavenging in carabids and staphylinids. Moreover, scavenging was also shown for a tetragnathid spider species, calling for further studies on scavenging. For the analysis of the microcosm and mesocosm experiments an advanced DNA-based gut content analysis was employed, enabling accurate evaluation of trophic links. To improve the applicability of these technique, laboratory feeding trials were conducted to investigate factors affecting prey DNA detectability in predator guts. Effects of ambient temperatures below 20 °C did not affect prey DNA detectability in predator guts, indicating minor importance of ambient temperature for DNA gut content analysis of soil or ground dwelling predators. Testing detection rates of four different amplicon sizes the largest and the shortest fragment significantly decreased and increased prey DNA detectability, respectively. Therefore, not only predation rates but also determination of the time when predation occurred in the field could be possible to some extent using primer pairs amplifying fragments differing distinctly in amplicon size. However, comparisons between predator species concerning predation rates have to be carefully interpreted because comparing detection rates of prey DNA between predator species revealed significantly differences in prey DNA detectability even between two closely related carabid species. Therefore, for accurately determination of predation rates of field caught predators through DNA-based gut content analysis, factors affecting DNA decay have to be considered as well as scavenging, producing ‘false positives’ if only predation is asked.

The present work documents the improvement of biological control by detrital subsidies, complementary predation of alternative and pets prey by generalist predators and indicates synergistic effects of rain and generalist predators on aphid predation. DNA-based predator gut content analysis proved to be highly valuable in investigating predator-prey interactions in simplified study systems. Ambient temperature, amplified fragment length and predator species identity demonstrated to affect prey DNA detectability in predator guts, and these factors as well as scavenging have to be considered in evaluating predation rates and thereby biological control effects of generalist predators in the field.

1 General Introduction

1.1. WHEAT AND ITS APHID PESTS

Wheat (*Triticum* spp. L.) is the most important cereal in the world, grown in all five continents. With 217 million hectares in 2005, no other cereal had more area under crops, gathering 630 million t of harvest in 2005 (FAO 2006). To guarantee high yields, pesticides are applied against weeds, fungi and herbivores. Among herbivores, cereal aphids are the most important pests in Europe and can cause high damage and yield loss due to directly damaging the plants but also by transferring viral diseases (Vickerman & Wratten 1979). The grain aphid *Sitobion avenae* F. (Aphididae) and the bird cherry-oat aphid *Rhopalosiphum padi* L. (Aphididae) are the dominant species in wheat, infesting both shoots and ears. The preferred feeding site of *S. avenae* is the ear (Wratten 1975), where it feeds predominantly on the rachis and on the base of the spikelets, which usually leads to substantial yield loss. Additionally, effects of honeydew can result in earlier senescence of the flag leaf, thereby also damaging the plant (Wratten 1975; Rabbinge *et al.* 1981). Infesting predominantly the ears enables *S. avenae* to maintain itself longest in crops compared to other aphid species as the ear remains longer physiologically active than the leaf. Furthermore, feeding on ears results in high reproductive rates (Watt 1979), and *S. avenae* multiplies twice as fast on the ear as on the flag leaf (Vereijken 1979). In contrast to *S. avenae*, *R. padi* prefers the stem base and the lower leafs as feeding site (Dean 1974; Leather & Lethi 1982). This spatial within-plant distribution of *R. padi* has been suggested to be modified by the nutritional value of the plant tissue (Leather & Dixon 1981; Weibull 1987; Wiktelius *et al.* 1990), avoidance of extreme field temperatures (Wiktelius 1987) and the feeding behaviour of polyphagous predators (Gianoli 1999).

In addition to the differences in feeding sites, *S. avenae* and *R. padi* also differ in their life cycles. Generally aphids show cyclical parthenogenesis, i.e. they reproduce both sexually and asexually, with several generations of apomictic parthenogenesis between each period of sexual reproduction. In addition to cyclical parthenogenesis, some aphid species (mainly in the family Aphididae) show host alternation (heteroecy). This usually involves migration between a woody host, on which sex occurs and eggs are laid and an herbaceous plant, where the reproduction is solely parthenogenetic. While *S. avenae* is monoecious on grasses (Poaceae), *R. padi* is heteroecious between roses (Rosaceae) and grasses. In both species parthenogenetic females (fundatrix) hatch from eggs and produce aptere offspring. Later in spring, winged (alate) migrants are produced, infesting cereals by leaving the primary host in case of *R. padi* or leaving other grassy hosts in case of *S. avenae*. Arriving in cereals, either apterae or alatae are produced. During summer alate migrants disperse further between the host plants. In autumn winged gynoparae are produced, giving birth to males and mating females. In *R. padi* the winged aphids (gynoparae and males) reinvade the primary host where also mating takes place. The mating females, also called oviparae, lay few eggs which overwinter.

In wheat fields the typical population development of aphids during summer consists of initial slow build-up, rapid multiplication, slow down, stagnation and rapid decrease (Vereijken 1979). In *S. avenae* summer populations usually develop from winged migrants but also from resident overwintering virginoparae. Rapid multiplication is partly achieved as larger embryos inside adult aphids have also embryos developing within them, already achieving most of their embryonic growth during their parents' development (Dixon 1987); thereby reducing the time until the new generation can produce offspring. Population density of grain aphids may double in three days and increase 50-fold in 20 days (Grünbacher *et al.* 2006). Summer dispersal within wheat is triggered by crowding in both *S. avenae* and *R. padi*, initiating the production of offspring developing into alatae (Dixon & Glen 1971; Watt & Dixon 1981). Take-off, and thereby dispersal, may be delayed by abiotic factors such as increasing wind-speed and decreasing temperatures. Dispersal over smaller distances can also occur in apterae, often triggered by rain, dislodging aphids from plants or initiating inter-plant

movement (Dhaliwal & Singh 1975; Zuniga 1991; Mann *et al.* 1995; Narayandas & Alyokhin 2006).

Generally, predicting aphid outbreaks in cereals is difficult, as a number of abiotic and biotic factors modulate aphid population dynamics. Aphid outbreaks have been recorded since the early 1970s, and have been related to increased applications of nitrogen fertilizers in combination with applications of fungicides and growth regulators (Hanisch 1980; Ankersmit 1988; Honek 1991). In addition, weather is suggested to significantly modulate probabilities of aphid outbreaks. Aphids survive better during mild winters (Dewar & Carter 1984), theoretically fostering aphid outbreaks. But winter weather also affects the growth of winter wheat, and harsh winters delay the development of wheat, thereby increasing the time span for cereal aphids to feed on wheat plants (Dixon 1998). Therefore, the likelihood of an aphid outbreak increases after harsh winter conditions if several mild winters preceded, probably resulting in high proportions of overwintering aphids (Dixon 1998). Further, specialist and generalist predators significantly affect aphid populations in wheat, thereby possibly preventing aphid outbreaks (Wratten & Powell 1991; Sigsgaard 2002; Symondson *et al.* 2002).

1.2. GENERALIST PREDATORS

Generalist predators feed on a wide range of prey and besides being mainly carnivorous, they may also feed on plants and fungi. The typical generalist predator feeds on almost anything it can subdue; however, most predators are restricted physiologically, behaviourally or physically to some degree in their prey choice. As generalist predators have to handle their (living) prey, body size is a powerful determinant of the prey range of a predator (Sabelis 1992). Still, knowledge on the diet breadth of most species is restricted, and to assign particular predator species to a category (stenophagous, oligophagous, polyphagous) is an uncertain process (Symondson *et al.* 2002). The development of molecular techniques to determine quantitatively and qualitatively the diets of predators offers new possibilities for uncovering trophic links especially in generalists (*see* Chapter 1.4).

Beside trophic interactions between trophic levels, also trophic interactions also exist within the predator trophic level. The latter can occur between different species (intraguild predation; IGP) or within the same species (cannibalism). Both mechanisms can disrupt the ability to control prey populations (Polis & Strong 1996; Snyder & Ives 2001). As species using the same resources kill and/or prey on each other, IGP limits interspecific competition and cannibalism limits intraspecific competition (Polis & Holt 1992). Both mechanisms, IGP and cannibalism, are common in terrestrial food webs (Polis *et al.*, 1989; Polis 1991) and have been shown to alter predator-prey interactions in agricultural systems (Snyder & Wise 2001; Lang 2003; Wise 2006). Further factors regulating generalist predator populations in arable systems are management practices, crop types and farming system. Among generalist predators three groups are most common in agricultural systems: carabids (Carabidae), staphylinids (Staphylinidae) and spiders (Araneae).

1.2.1. Coleoptera, Carabidae

Carabids are relatively easy to observe and to collect and have been studied extensively throughout the 20th century. The carabid community inhabiting agricultural fields is dominated by almost the same species in whole Europe (Thiele 1977). Carabids are sensitive to changes in habitat structure due to management practices and crop rotation and are therefore used as bioindicators (Lövei & Sunderland 1996). Generally, the larvae are more vulnerable to mechanical disturbances, such as ploughing, than the adult beetles. Reproduction rhythms in carabids differ between species. Generally two types are distinguished: (i) species with winter-larvae, with eggs being laid in late summer or autumn, and (ii) species with summer larvae, with eggs being laid in spring (Den Boer & Den Boer-Daanje 1990). However, many carabid species are flexible in their development, hampering classifications of reproductive types (Lövei & Sunderland 1996). The reproductive type strongly controls the activity rhythm of the beetles, and therefore most carabid species show one or two activity peaks in spring/early summer and/or in late summer/autumn, which does not exclude carabid activities during winter. Carabids are very mobile, mostly by moving at high speeds at the soil surface, but also, mainly in smaller species, by flying (Holland 2002). Prey detection mainly functions through touching prey (tactile cues), followed by attacking

the target (Wheater 1989). However also vision (mainly in diurnal generalist carabids) and olfactory cues from the prey, detected by the antennae, help localising the prey. Some carabids have been found being attracted by aphid alarm pheromones, despite the fact that aphids are of low food quality or even toxic for carabid beetles (Toft 2005).

1.2.2. Coleoptera, Staphylinidae

Staphylinids are not easy to determine to species level, which is probably one explanation for the few studies being available investigating these beetles. Staphylinids are very abundant in arable fields and, like in carabids, few species dominate the community structure (Krooss & Schaefer 1998). The community and diversity of staphylinids reflects management practices with higher diversity in fields receiving reduced tillage and fertiliser application. Most species are able to fly and therefore staphylinids are very mobile. They can act as detritivores, herbivores or predators, and some species are known as parasites (Fournet *et al.* 2000). They are active aboveground, where some species have been shown to prey on aphids, and also belowground, where they prey on collembolans, feed on algae, fungi and decaying organic matter (Good & Giller 1991). Therefore, like carabids, staphylinids act as link between the belowground and the aboveground system.

1.2.3. Araneae

Spiders are abundant in most agroecosystems (Riechert & Lockley 1984), mostly feeding exclusively on arthropods (Wise 1993). Due to their foraging behaviour spiders are ascribed to two functional groups, web-builders and cursorial spiders; the latter hunting their prey without building webs (Uetz 1992; Wise 1993). Web-building spiders mostly rely on insect prey whereas cursorial spiders also prey on other spiders (Nyffeler 1999). In temperate European agroecosystems, the spider fauna is dominated by sheet-web weavers (Linyphiidae) and wolf spiders (Lycosidae) (Nyffeler & Sunderland 2003). While linyphiids rely on prey captured in their web or sometimes also hunt for prey (Alderweireldt 1994), lycosids range from sit-and-wait predators that ambush prey to more active predators that hunt down their prey (Marshall *et al.* 2002). Lycosids are visually orientated, detecting their prey by

movement and vibrations (Uetz 1992; Samu 1993; Persons & Uetz 1998). Both, linyphiids and lycosids are known to include aphids and collembolans in their diet (Nyffeler & Benz 1988; Agusti *et al.* 2003; Harwood *et al.* 2004). Dispersal of most agrobiont spiders happens through aerial drifting (ballooning, Weyman *et al.* 2002), for which spiders climb to exposed parts of the vegetation and use silk strands to get drifted by the wind.

1.3. BIOLOGICAL CONTROL

Biological control is defined as the action of predators, parasitoids, pathogens or hormones suppressing pest populations to lower levels than would have occurred without them. Biological control can occur naturally or can be induced by man. The 'classical biological control' mainly deals with specialist natural enemies, especially parasitoids. Specialists have been seen for a long time as the perfect biocontrol agent as they are highly prey or host specific (DeBach & Rosen 1991; Hoy 1994), with their life cycle being adapted to that of their target. Reviewing data from biological control and insect life table literature, Hawkins *et al.* (1999) concluded that successful top-down control is most frequently due to a single specialist species for exotic insect herbivores on exotic plants in simplified, cultivated habitats, whereas control of native herbivores on native plants in natural habitats is more frequently due to communities of generalist predators. However, in highly disturbed or temporary agroecosystems often mobile, opportunistic pest species predominate and in such habitats natural enemies with similar ecological strategies are required (Ehler 1998). Effective natural enemies in such habitats should have (i) colonising ability to keep pace with temporal and spatial disruptions, (ii) temporal persistence, also in times when pest prey is scarce, and (iii) opportunistic feeding habits that allow the predator to rapidly exploit the arriving pest prey (Ehler 1990). Especially the last two are characteristic for generalist predators, and there is growing evidence that naturally occurring generalist predators can suppress pest populations in agricultural systems, thereby reducing yield loss (reviewed in (Symondson *et al.* 2002).

The term 'conservation biological control' combines pest suppression by an already established natural enemy community and the manipulation of the environment (i.e. management practices) to foster the survival and/or the physiological and behavioural performance of these enemies (Barbosa 1998). Besides intercropping, weed strip management or fallow management, one technique to foster generalist predators is through increasing alternative prey availability. Microbi-detritivores are potentially alternative prey for generalist predators, and it has been stressed by Polis (1994) and Polis and Strong (1996) that periodically feeding on prey from the decomposer system may strengthen herbivore control by generalist predators ('dual subsystem omnivory'; (Scheu 2001). Decomposer organisms generally appear to be limited by the availability of dead organic matter (Hairston *et al.* 1960; Hairston 1989; Scheu & Schaefer 1998), allowing to engineer the detrital food web through application of allochthonous resources. However, this method might fail if predators do not switch from alternative prey to pest prey when this arrives. Yet only few studies investigated the effect of allochthonous resources on pest suppression (Settle *et al.* 1996; Halaj & Wise 2002), and results are contradictory. Elevated densities of generalist predators might also result in increased inter-trophic predation (IGP, cannibalism; *see* Chapter 1.2.), and this may also temper biological control. All these factors (IGP, cannibalism and switching prey) are rooted in the polyphagous habit of generalist predators; therefore evaluating trophic links is essential to understand the role generalist predators might play in biological control.

1.4. INVESTIGATING TROPHIC LINKS: STABLE ISOTOPE ANALYSIS AND MOLECULAR TECHNIQUES

Investigation of trophic links is inevitable for the understanding of ecological processes shaping animal populations. Field monitoring of animal communities to study multitrophic interactions might be possible in vertebrates, and sometimes also in invertebrates (e.g. video techniques; Meyhofer 2001), but is virtually impossible in species being small, nocturnal or living subterranean. Those species can be sampled from the field with a large variety of methods, such as pitfall traps, sweep nets, sticky traps, malaise traps, hand searching, suction sampling or by knock-down (Sunderland *et al.* 2005), and sampled species can be post-

mortem analysed for their diets. This is highly advantageous as the system under study is not disturbed prior to collection and animals can be assumed have been acting naturally (Sunderland 1988; Symondson 2002). Post-mortem analyses include, amongst others, body tissue analysis and gut content analysis.

1.4.1. Stable Isotope analysis

Carbon and nitrogen, main components of living bodies, have more than one stable isotope, and many biological processes are accompanied by changes in the ratio between these stable isotopes ($^{12}C/^{13}C$ and $^{14}N/^{15}N$). Because the variation within the isotopic composition in natural materials is rather narrow it is commonly expressed in ppm difference by comparison with the international standard:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = (R_{sample} - R_{standard})/(R_{standard} \times 1000)$$

where R_{sample} and $R_{standard}$ represent the ^{13}C-to-^{12}C or ^{15}N-to-^{14}N ratio in samples and standard, respectively. Significant in ecological studies are carbon isotope fractionation during photosynthesis and ^{15}N accumulation in trophic levels (Tiunov 2007).

Compared to the standard for carbon (Pee Dee Belemnite), atmospheric CO_2 has a $\delta^{13}C$ value of about -8‰. During diffusion through the plant tissue into the internal gas space CO_2 is depleted in the heavier isotope by ~4‰. Subsequently, carbon fixing related enzymes further discriminate against the heavier isotope. As the initial carboxylating enzymes differ between C3 and C4 plants, these plants strongly differ in their carbon stable isotope ratios. In C3 plants with the carboxylating enzyme ribulose bisphosphate carboxylase (rubisco) median $\delta^{13}C$ values are about -27‰, whereas in C4 plants with the initial carboxylating enzyme phosphoenolpyruvate (PEP) carboxylase median $\delta^{13}C$ values are less negative and cluster to about -14‰ (Lajtha & Marshall 1994). These differences in carbon isotope ratios of C3 and C4 plants have been used to trace carbon fluxes and determine the fractions of different food sources in animal diets (Fry *et al.* 1978; Boutton *et al.* 1983; Martin *et al.* 1992). Particularly labelling the soil food web with a resource (C4 plant litter, e.g maize) differing in their $\delta^{13}C$ signature from the resource aboveground (C3 plant, e.g. rye, barley, wheat) is a promising

tool to investigate the diets of organisms linking these two subsystems, e.g. by generalist predators.

Heavy nitrogen accumulates in food chains (Miyake & Wada 1967; Minagawa & Wada 1984), which is supposed to be due to the discrimination of the ^{15}N isotope in the synthesis of excreted nitrogen metabolites (Macko *et al.* 1986). The enrichment in ^{15}N per trophic level is about 3 ‰ (DeNiro & Epstein 1981; Minagawa & Wada 1984; Wada *et al.* 1991; Post 2002), but variations exist as enrichment depends on the diet, nitrogen secretion pattern and taxonomic group (reviewed in Tiunov 2007). Furthermore, trophic enrichment can depend on the hunger/starvation level, animal age and life cycle stage (Ponsard & Averbuch 1999; Oelbermann & Scheu 2002; Scheu & Folger 2004; Haubert *et al.* 2005). Despite these variations $\delta^{15}N$ signatures correctly reflects the trophic level of e.g. soil animals (Tiunov 2007), and has been used to analyse meso- and macrofauna food webs in forests (Scheu & Falca 2000) and arable land (Albers *et al.* 2006). As stable isotope signatures reflect the main food source of consumers over a larger time span, a shortcoming of this method is the inability to determine current shifts in diets as well as exact information of ingested organisms on species level.

1.4.2. Molecular techniques

The easiest technique in analysing gut contents of insects is visual examination (Ingerson-Marhar 2002; Sunderland *et al.* 2005), but this technique only allows to trace prey leaving solid food remains in the gut of the predator. Generalist predators, however, are mostly fluid feeders or simply may avoid consuming hard, indigestible prey remains and therefore visual gut examination may miss many trophic interactions (Dennison & Hodkinson 1983). Molecular techniques do not have such restrictions, and several approaches including protein electrophoresis, monoclonal and polyclonal antibodies, and DNA-based techniques, have been developed, all afflicted with specific assets and drawbacks (reviewed in Symondson 2002; Sheppard & Harwood 2005; Sunderland *et al.* 2005). Monoclonal antibodies are sensitive and highly specific and have been used for detection of single prey species in predators. Due to high costs in both, facilities and developing time, this technique, however,

is barely applicable for generalist predators exploiting a large number of different prey species (Symondson 2002).

Recently, DNA-based techniques have been rapidly developed overcoming some restrictions existing in monoclonal antibodies. Sequences of many target species are already available from phylogenetic studies, and primers, once being developed and published, can easily adopted by other researchers. Degradation of prey materials in predator guts are a problem in both, antibody and DNA techniques, but sensitivity can be increased in the latter due to amplification of any intact DNA employing a PCR step. However, effects on degradation of DNA in predator guts, and thereby on prey DNA detection success, have to be considered for interpreting field data and assess trophic links and their strength in natural systems. In general, DNA is spontaneously decayed due to hydrolysis, oxidation and nonenzymatic methylation (Lindahl 1993). In predator guts digestive enzymes presumably accelerate DNA decay, and digestion time has been shown to significantly decrease DNA detectability (Chen *et al.* 2000; Harper *et al.* 2005; Juen & Traugott 2005, 2006, 2007; Ma *et al.* 2005; Sheppard *et al.* 2005; Greenstone *et al.* 2007). Also the amplified fragment length affects DNA detectability, with longer fragments being less traceable than short ones (Agusti *et al.* 1999; Zaidi *et al.* 1999; Hoogendoorn & Heimpel 2001; Agusti *et al.* 2003). Additionally, ambient temperature seems to affect detection success (Hoogendoorn & Heimpel 2001), and some of the former effects have been shown to vary between different predator taxa (Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Ma *et al.* 2005; Read *et al.* 2006; Greenstone *et al.* 2007). Besides knowledge about DNA decay, it is also essential to gain information about the way prey material has entered predator guts. This information is fundamental if predation and not only consumption is of interest, especially to determine biological control effects of generalist predator species. DNA-based techniques are not able to distinguish between prey ingestion of living or dead (scavenging) prey (Foltan *et al.* 2005; Juen & Traugott 2005). As the consumption of dead (or also moribund) prey has no pest

control value (Sunderland 1996), 'false' positives in predator screenings would lead to wrong estimates of predation rates. In contrast, results from DNA-based methods suggest that secondary predation, where a predator consumed another predator which consumed prey, is of minor significance (Sheppard *et al.* 2005). Furthermore, until now, detection of prey DNA is only qualitatively, without any information about the quantity of consumed prey. However, first experiments using quantitative real-time PCR could quantify the amount of prey DNA of several species in predator's faeces, possibly promoting DNA-based techniques towards quantitative diet composition analyses (Deagle & Tollit 2007).

A further promising step in DNA-based gut content analysis is the adoption and further development of multiplex PCR, which allows to amplify many targets of interest in one reaction by using more than one pair of primers. This method, first described by Chamberlain *et al.* (1988), can be tedious and time-consuming to establish, but saves time and costs once installed. Multiplex PCR has been used recently in generalist predator gut content analyses and proved to be highly valuable in multiple prey screening (Harper *et al.* 2005) as well as false-negative identification through simultaneously amplification of both predator and prey DNA (Juen & Traugott 2006, 2007).

1.5. OBJECTIVES

The present work investigated the effect of generalist predators on aphid populations in winter wheat. To obtain results relevant for farming practice, two field studies, of which one was replicated within and between fields, were conducted on areas of the Reinshof research farm of the University of Göttingen. These field experiments dealt with alternative prey availability, prey density and resource availability affecting aphid predation by generalist predators. In two microcosm/mesocosm studies, the mechanisms driving predator-prey interactions in winter wheat systems were investigated in more detail, including abiotic factors and carrion consumption. In both studies DNA-based molecular gut content analyses were employed. To allow more straightforward interpretation of results from these studies and

to advance the applicability of PCR gut content analysis, laboratory feeding experiments were performed to evaluate factors modifying DNA detectability in predator guts including ambient temperature, amplified fragment length, digestion time and predator species identity.

CHAPTER 2: In a field experiment the effects of maize mulch on generalist predators and their ability to control aphid populations were investigated. Since generalist predators feed on both, the decomposer system and the herbivore system, we hypothesised that application of an allochthonous resource (maize mulch) will increase decomposer densities, thereby increasing generalist predator densities due to higher prey availability. We further hypothesized that increased predator densities have a negative effect on aphid populations, and that this trophic cascade is consistent in different agricultural fields.

CHAPTER 3: In a second field experiment the effects of a generalist predator guild on aphid populations at different aphid densities was evaluated. Generally, aphid suppression is supposed to be strongest at low aphid densities. However, results from our first field experiment (Chapter 1), suggested that aphids may also be controlled at high densities. Therefore, we established low, medium and high initial aphid densities inside field cages, hypothesising that the natural community of generalist predators has also control effects on aphids at high densities, and that these effects are not disrupted by the presence of alternative prey.

CHAPTER 4: In a microcosm experiment the effect of rain on predator-prey interactions in a simplified winter-wheat system was investigated. We hypothesised that rain dislodges aphids from the wheat plants. As a consequence, prey availability on the soil surface should increase, causing synergistic effects of rain and ground-dwelling predators on aphid populations. To evaluate changes in aphid predation rates directly after rain, a fraction of the predators was removed and predator guts were analysed for aphid DNA.

CHAPTER 5: In a mesocosm experiment preferences for either dead or living prey in a guild of generalist predators, including carabids, staphylinids and spiders, were evaluated. To predict the strength of trophic links and their implications for prey populations, consumption pathways, e.g. predation, scavenging, secondary predation or consumption of moribund prey, needs to be considered. Especially scavenging is supposed to be common in generalist predators, thereby tampering evaluations of predation rates by gut content analysis, as these techniques can not differentiate between predation and scavenging. We developed a multiplex PCR approach to detect DNA of two different aphid species, introduced into mesocosms as either living or dead prey, in generalist predators. We hypothesised that single predator species show preferences for one of the prey types. Furthermore, we expected that predators feeding on dead aphid prey to be rather ground searching and predators feeding on living prey to be rather foliar foraging.

CHAPTER 6: In laboratory feeding experiments factors modifying prey DNA detection rates in two carabid beetle species were investigated. Knowledge of factors affecting detectability of prey DNA in predator guts is crucial for interpretation of molecular gut content analyses derived data from field caught predators. We hypothesised that DNA breakdown and therefore detection times in predator guts are decreased by increasing ambient temperature, digestion time and length of the targeted fragment. Furthermore, we expected significant differences in prey DNA detectability between two closely related carabid species.

2 | Detrital subsidies increase herbivore control in cereal fields by fostering the impact of individual predator species

2.1. ABSTRACT

Prey from the decomposer subsystem can support predator populations in arable fields, and fostering alternative prey through adding detrital subsidies to agricultural systems potentially enhances pest control. We investigated whether resource addition (maize mulch) initiates trophic cascades in winter wheat fields. We evaluated the input of the maize-born carbon into the food web after nine months via stable isotope analysis, allowing to differentiate between prey originating from the above- and belowground subsystems in predator diets. Furthermore, we recorded aphid populations in predator reduced and control plots in no-mulch and mulched fields. All the analysed soil dwelling species had incorporated maize-born carbon with the contribution of maize born carbon to animal tissue carbon varying between 15 % and 65 %. In contrast, only two of 13 aboveground predator species had incorporated maize carbon, suggesting that these two predators forage on prey from the above- and belowground system. Densities of these two predator species were increased in the mulched fields. Interestingly, collembolan species differed markedly in their maize carbon signals. The endogeic genus *Onychiurus* incorporated 30 % maize-born carbon in contrast to the three epigeic collembolan species with no maize-born carbon incorporation. Accordingly, the addition of mulch did not affect the abundance of epigeic collembolans. Nitrogen isotope signatures suggest that the generalist predators in part fed on these collembolans; indeed, single predator species were more abundant in mulch treatments and this was associated by increased aphid suppression. Our results suggest that detrital subsidies easily enter belowground food webs, and certain

aboveground predator species are tightly linked to this system. These predators likely were responsible for aphid suppression in the mulch treatments. Therefore, engineering the decomposer subsystem via detrital subsidies offers the possibility to strengthen biological control by generalist predators feeding on both herbivores and microbi-detritivores.

2.2. INTRODUCTION

Generalist predators, including carabids, staphylinids and spiders, are among the most important predators in terrestrial systems (Krooss & Schaefer 1998; Lang *et al.* 1999; Scheu 2001). They can effectively decrease prey populations (Wise 1993; Lövei & Sunderland 1996), including pests in agricultural systems (Symondson *et al.* 2002). Due to their catholic feeding, they may lack attributes of the ideal biological control agent, such as prey specialisation. However, including non-pest prey in their diet may sustain generalist predator populations in fields when pest prey is scarce (Symondson *et al.* 2002), an important feature lacking in specialists. Microbi-detritivores are potentially alternative prey for generalist predators, and it has been stressed by Polis (1994) and Polis and Strong (1996) that periodically feeding on prey from the decomposer system may strengthen herbivore control by generalist predators. Decomposer organisms generally appear to be limited by the availability of dead organic matter (Hairston *et al.* 1960; Hairston 1989; Scheu & Schaefer 1998), allowing to engineer the detrital food web through application of allochthonous resources.

There is evidence that fostering the decomposer subsystem through detrital subsidies can lead to increased densities of generalist predators in rice (Settle *et al.* 1996) and vegetable garden systems (Halaj & Wise 2002). However, effects on pest populations or plant yield are contradictory, and several suggestions have been made about effects tempering strong trophic cascades (Halaj & Wise 2002). Increased densities of generalists may enhance intraguild predation and cannibalism. Both are common in terrestrial food webs (Polis *et al.* 1989; Polis 1991) and have been supposed to alter biological control (Snyder & Wise 2001; Lang 2003; Wise 2006). Secondly, generalist predators may fail to switch to pests in the presence of

highly abundant alternative prey. Especially aphids have been shown to be of low food quality or even toxic for generalist predators (Toft 2005), possibly preventing generalist predators to substitute the more palatable alternative prey with pests. For a better understanding of the factors affecting pest suppression, information on trophic links and food web structure of above- as well as belowground arable systems is needed.

The analysis of natural variations in stable isotopes holds a promising tool to investigate trophic interrelationships, especially in food webs where polyphagous predators dominate (Ponsard & Arditi 2000; Scheu & Falca 2000; Scheu 2002). The concentration of ^{15}N in consumers increases at higher trophic levels, thereby allowing to determine the trophic position of species in food webs (DeNiro & Epstein 1981; Minagawa & Wada 1984; Post 2002). In contrast to ^{15}N, carbon isotopes are little fractionated in consumers (Peterson & Fry 1987; Wada *et al.* 1991), thereby allowing to determine food resources of consumers. Differences in carbon isotope ratios of resources have been used to trace carbon fluxes and determine the fractions of different food sources in animal diets (Fry *et al.* 1978; Boutton *et al.* 1983; Martin *et al.* 1992). C4 and C3 plants markedly differ in their $^{13}C/^{12}C$ ratio, and maize (C4 plant) as a food resource for the decomposer subsystem has been used before to trace carbon fluxes into the soil food web of arable systems (Albers *et al.* 2006). They found the maize carbon to be rapidly incorporated into the decomposer system; however, further carbon fluxes into the aboveground subsystem were not evaluated.

In the present study we investigated the effect of a detrital subsidy (maize mulch) on the decomposer subsystem and the feedbacks on the aboveground generalist predator - herbivore system in winter wheat. To allow general conclusions three wheat fields embedded in different landscapes were investigated. We hypothesised that (i) an allochthonous resource (maize mulch) boosts decomposer densities with effects reaching into the next growing season; (ii) generalist predators feed on prey belonging to the decomposer subsystem, resulting in increased predator densities in mulched fields and, if the previous assumption is true, (iii) increased densities of generalist predators suppress herbivore populations.

2.3. MATERIALS AND METHODS

Study site

The experiment was conducted between September 2003 and August 2004 in three winter wheat fields managed by the Reinshof research farm of the University of Göttingen (Lower Saxony, Germany). Two fields are located near the River Leine at 150 m above sea level. Field 1 is surrounded by cereal and root crop fields; field 2 is close to the city of Göttingen, adjacent to a garden colony. The soil is a loamy flood-plain soil consisting on clayey silt. Field 3 is located in the north of Göttingen at 320 m above sea level with a shallow soil (Rendzina) over shell limestone. It is surrounded by hedgerows and groves, embedded in a diverse landscape of a mixture of forests, hedgerows and pastures. The mean annual temperature in Göttingen is 8.7 °C, and the mean annual precipitation is 645 mm. Mean temperature was higher than average during both years of the study, mean rainfall was lower in 2003 and higher in 2004 than average (9.4 °C and 550 mm in 2003; 9.1 °C and 718 mm in 2004).

Experimantal setup and sampling

In each field two randomly chosen areas of 1 ha each received 15 t (wet weight) of maize chaff in September 2003. The maize chaff was equally distributed within the 1 ha areas and homogenised with the upper soil layer through grubbing. Subsequently, all three fields including the mulched areas were sown with winter wheat. In May 2004 experimental treatments were established in a $2 \times 2 \times 2$ factorial design with the factors 'mulch' (with and without), 'ground dwelling predators' (GP; reduced and open control) and 'flying predators' (FP; reduced and open control) with two replications per field. To reduce ground dwelling predators, plastic barriers were installed reaching 10 cm into soil and 40 cm aboveground enclosing a circular area of 2 m². Four 'live' pitfall traps (without trapping liquid) situated at the inner edge of the barriers were established in a cross design. Pitfalls were cleared daily over a period of 19 days throughout June. All predators, i.e. carabids, staphylinids and lycosids, were visually identified, counted and released outside the plots. All other animals were returned inside the plots. To reduce flying aphid predators and parasitoids wire cages

(mesh size 8 mm) were set over the plots at the end of June. Cages were sprayed with non-toxic glue (Soveurode Aerosol, Witasek, Austria) to inhibit or capture flying arthropods.

In each plot one flowerpot (Ø 25 cm) filled with potting soil and planted with wheat of the same variety as in the fields was buried with the edging of the pot at ground level enabling surface active arthropods to access the pots. The pots were established to test for direct mulch effects on aphid population development, e.g. due to changes in nutrient availability. Aphids as well as predators were counted visually on 25 wheat shoots per plot and per pot, respectively, at wheat flowering (end of June) before the installation of the wire cages and at milk ripening after the cages had been removed (mid of July).

To determine the densities of ground dwelling arthropods, such as generalist predators and surface active collembolans, plastic barriers as described above were installed in all three fields in both the mulch and no-mulch treatments and replicated four times. Four pitfall traps containing an oversaturated saltwater solution were established in a cross design at the inner edge of the barriers. The pitfall traps operated for two weeks on three dates, respectively (26 May-9 June; 23 June-7 July; 21 July-4 August). During dates when pitfall traps were not operating they were closed and the plastic barriers were lifted 15 cm above ground to enable recolonisation by arthropods. Additionally, one soil core (diameter 21 cm) adjacent to each of the plots was taken at the same dates pitfall traps were opened. Soil animals were extracted from the soil cores by stepwise increasing heat (Kempson *et al.* 1963). Invertebrates from the pitfall traps as well as from the soil cores were determined to genus or species level, counted, and stored in oversaturated saltwater solution at -10 °C until they were processed for stable isotope analysis.

^{13}C and ^{15}N analysis

Wheat plants from the experimental plots as well as maize chaff were dried at 60 °C for two days, ground and dried again at 60 °C for one day. Samples of ~ 2.6 mg dry mass were prepared for ^{13}C and ^{15}N analysis. Animals were washed in distilled water and dried at 60 °C for six days. Either homogenised animal tissue or whole animals (80 - 1660 µg) were used for stable isotope analysis. In mesofauna species two or more specimens were bulked per sample

to reach appropriate sample weight. All samples were kept in a desiccator until mass spectrometer analysis.

Samples were analysed by a coupled system consisting of an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) and a gas isotope mass spectrometer (MAT 251, Finnigan, Bremen, Germany). The system is computer controlled allowing online measurement of ^{13}C and ^{15}N (Reineking *et al.* 1993). As primary standards for the isotope values of carbon and nitrogen Pee Dee Belemnite (PDB) limestone and atmospheric air were used, respectively. Acetanilide (Merck, Darmstadt, Germany) was used for internal calibration. Isotope natural ratios were expressed using the delta notation with $\delta^{13}C$ or $\delta^{15}N$ (‰) = $(R_{sample} - R_{standard})/(R_{standard} \times 1000)$ where R_{sample} and $R_{standard}$ represent the ^{13}C-to-^{12}C or ^{15}N-to-^{14}N ratio in samples and standard, respectively. Incorporated maize-born carbon in animals tissue (x_m) was calculated by a two-source mixing model (Ludlow *et al.* 1976; Martin *et al.* 1992; Lepage *et al.* 1993) with x_m (%) = $(\delta_{am} - \delta_{aw})/\delta_{pm} - \delta_{pw}) \times 100$; δ_{am} and δ_{aw} refer to the $\delta^{13}C$ signature of an animal taxon in the plots with and without maize-mulch, respectively, and δ_{pm} and δ_{pw} to the $\delta^{13}C$ signature of the maize-mulch and the wheat plants, respectively.

Statistical analysis

Data on densities of ground dwelling predators and collembolans were analysed by repeated measures (RM) ANOVA with the fixed factors ‘field’ (1, 2, 3) and ‘mulch’ (with, without) at the three consecutive sampling dates. Univariate analyses were performed as it has been stated to be critical to perform RM MANOVA at small sample sizes (Maxwell & Delaney 1990). Furthermore, the RM MANOVA has been shown to be less powerful than the univariate counterpart (Cole & Grizzle 1966; Potvin *et al.* 1990). Within-subject probabilities were Huynh-Feldt corrected as this adjustment has been shown to perform well in terms of type I error and power (Stiger *et al.* 1998) and is recommended by Potvin *et al.* (1990). In case of significant interactions between one or both of the fixed factors and sampling date, two-way ANOVAs were calculated to analyse the effects of ‘field’ and/or ‘mulch’ at separate sampling dates. Where appropriate, Bonferroni corrected post-hoc tests were performed to identify differences between treatments. In case of the significant factor sampling date, also Bonferroni corrected post-hoc tests were performed to identify differences between dates.

Data on aphid densities were analysed by five-factorial analysis of variance (ANOVA) with the dependent variable 'aphids' (numbers per shoot) and the independent variables 'pot' (yes, no), 'field' (1, 2, 3), 'mulch' (with, without), 'ground dwelling predators' (GP; reduced and control) and 'flying predators' (FP; reduced and control). Data were square root transformed prior to the analyses. Where appropriate, Tukey´s HSD was used for multiple post-hoc comparisons.

Data on $\delta^{15}N$ and $\delta^{13}C$ signatures were analysed by one-way ANOVAs with the independent variable 'mulch' using the GLM procedure to account for unequal cell sizes. All statistical analyses were performed using STATISTICA 7.1 (StatSoft, Tulsa, USA).

2.4. RESULTS

Soil animals

From soil cores the following species were extracted: *Pergamasus* sp. Berlese (Mesostigmata); *Blaniulus guttulatus* F. (1798), *Brachyiulus pusillus* Leach (1815), *Polydesmus inconstans* Latzel (1884) (Diplopoda); *Lithobius microps* Meinert (1868), *Necrophloeophagus longicornis* Leach (1858) (Chilopoda); Symphyla; *Onychiurus* spp. Gervais (1841) (Collembola); elaterid larvae (Coleoptera); sciarid larvae and tipulid larvae (Diptera). Densities of all these taxa were too low for statistical analysis, therefore individuals were pooled for sample dates and in some taxa also for fields, but kept separated for the mulch treatments and used for stable isotope analysis.

Ground-dwelling predators

With the 'live' pitfall traps 728 rove beetles, 376 carabids and 87 lycosids were removed from the reduced ground-dwelling predator and the reduced flying and ground dwelling predator treatments during 19 days throughout June. On average, ratios between predator numbers removed from mulch and no-mulch plots were 64:36 in rove beetles, 60:40 in carabids and 31:69 in lycosids.

With the saltwater filled pitfall traps 1094 carabids (34 species), 180 carabid larvae, 509 staphylinids (12 genera) and 353 staphylinid larvae (5 subfamilies) were captured. As staphylinids were affected by sampling date, field and mulch (RM-ANOVA; date × field × mulch: $F_{4,36} = 3.34$, $P = 0.019$) and carabids by sampling date and field (RM-ANOVA; date × field: $F_{4,36} = 11.09$, $P < 0.001$), RM-ANOVAs were calculated for the most abundant staphylinid and carabid species.

In staphylinids, abundances of *Philonthus* spp. (mainly *Philonthus fuscipennis*) were affected by field and mulch irrespective of sampling date, with significantly higher abundances in mulch in field 1 at all three sampling dates (RM-ANOVA field × mulch: $F_{2,18} = 7.20$, $P = 0.005$) (Fig. 1a). Abundances of *Oxytelus inustus* were affected by sampling date and mulch (RM-ANOVA; date × mulch: $F_{2,36} = 5.47$, $P = 0.008$), with significantly higher abundances in the mulch plots in fields 2 and 3 at the first sampling date (one-way ANOVA; $F_{2,18} = 5.36$, $P = 0.015$) and still higher abundances in the mulch plots in field 3 at the second sampling date (one-way ANOVA; $F_{2,18} = 5.19$, $P = 0.017$; Fig. 1b). Abundances of staphylinid larvae differed between sampling dates and fields (RM-ANOVA; date × field: $F_{4,36} = 7.36$, $P < 0.001$), and were significantly increased in the mulch plots in all fields at the second sampling date (one-way ANOVA; $F_{1,18} = 10.14$, $P = 0.005$; Fig. 1c).

In carabids abundances of *Trechus quadristriatus* were significantly affected by sampling date, field and mulch (RM-ANOVA; date × field × mulch: $F_{4,36} = 2.73$, $P = 0.044$), with significantly higher abundances in the mulch plots in all fields at the first (one-way ANOVA; $F_{1,18} = 10.57$, $P = 0.004$) and second sampling date (one-way ANOVA; $F_{1,18} = 6.90$, $P = 0.017$). At the third sampling date, only in field 2 abundances were still significantly higher in the mulch plots (one-way ANOVA; $F_{2,18} = 4.00$, $P = 0.037$; Fig 1d). Data of abundances of *Notiophilus biguttatus*, *Notiophilus palustris*, *Loricera pillicornis* (Carabidae) and *Stenus sp.* (Staphylinidae) were pooled together and labelled as 'collembolan feeders' (Weinreich 1968; Thiele 1977). This group was affected by sampling date and mulch (RM-ANOVA; date × mulch: $F_{2,36} = 3.61$, $P = 0.037$). At the first sampling date, abundances of 'collembolan feeders' were only significantly higher in mulch plots in field 3 (one-way ANOVA; $F_{2,18} = 4.02$, $P = 0.036$), whereas at the second sampling date abundances were marginally significantly higher in mulch plots in field 1 and 3 but lower in field 2 (one-way

ANOVA; $F_{2,18} = 2.62$, $P = 0.10$; Fig 1e). Abundances of carabid larvae were affected by sampling date and field (RM-ANOVA; date × field: $F_{4,36} = 2.69$, $P = 0.046$), but also by mulch with significantly higher abundances in mulch plots in all three fields at the first sampling date (one-way ANOVA; $F_{1,18} = 17.05$, $P < 0.001$; Fig 1f).

Collembola

With pitfall traps 29,447 *Lepidocyrtus cyaneus*, 25,011 *Isotoma viridis* and 2,516 *Entomobrya lanuginosus* were captured in all three fields during the three consecutive trapping periods. Abundances of *Lepidocyrtus cyaneus* were affected by sampling date and mulch (RM-ANOVA; date × mulch: $F_{2,36} = 6.32$, $P = 0.004$), with lower abundances in mulch plots in field 2 being marginally significant at the first (one-way ANOVA; $F_{2,18} = 3.42$, $P = 0.055$) and the second sampling date (one-way ANOVA; $F_{2,18} = 2.86$, $P = 0.083$) and significant at the third sampling date (one-way ANOVA; $F_{2,18} = 15.61$, $P < 0.001$; Fig. 1g). Abundances of *Isotoma viridis* were significantly affected by sampling date, field and mulch (RM-ANOVA; date × field × mulch: $F_{4,36} = 4.89$, $P = 0.003$) with significantly higher abundances in mulch plots in field 1 at the first sampling date (one-way ANOVA; $F_{2,18} = 8.37$, $P = 0.003$) but significantly lower abundances in mulch plots in field 2 at the second (one-way ANOVA; $F_{2,18} = 4.55$, $P = 0.025$) and third sampling date (one-way ANOVA; $F_{2,18} = 12.65$, $P < 0.001$; Fig. 1h). Abundances of *Entomobrya lanuginosa* were significantly affected by sampling date and field (RM-ANOVA; date × field: $F_{4,36} = 7.86$, $P < 0.001$), with abundances in mulch plots being significantly higher in field 1 but lower in field 2 at the first sampling date (one-way ANOVA; $F_{2,18} = 25.14$, $P < 0.001$) and no significant differences between mulched plots at the second and third sampling date (Fig. 1i).

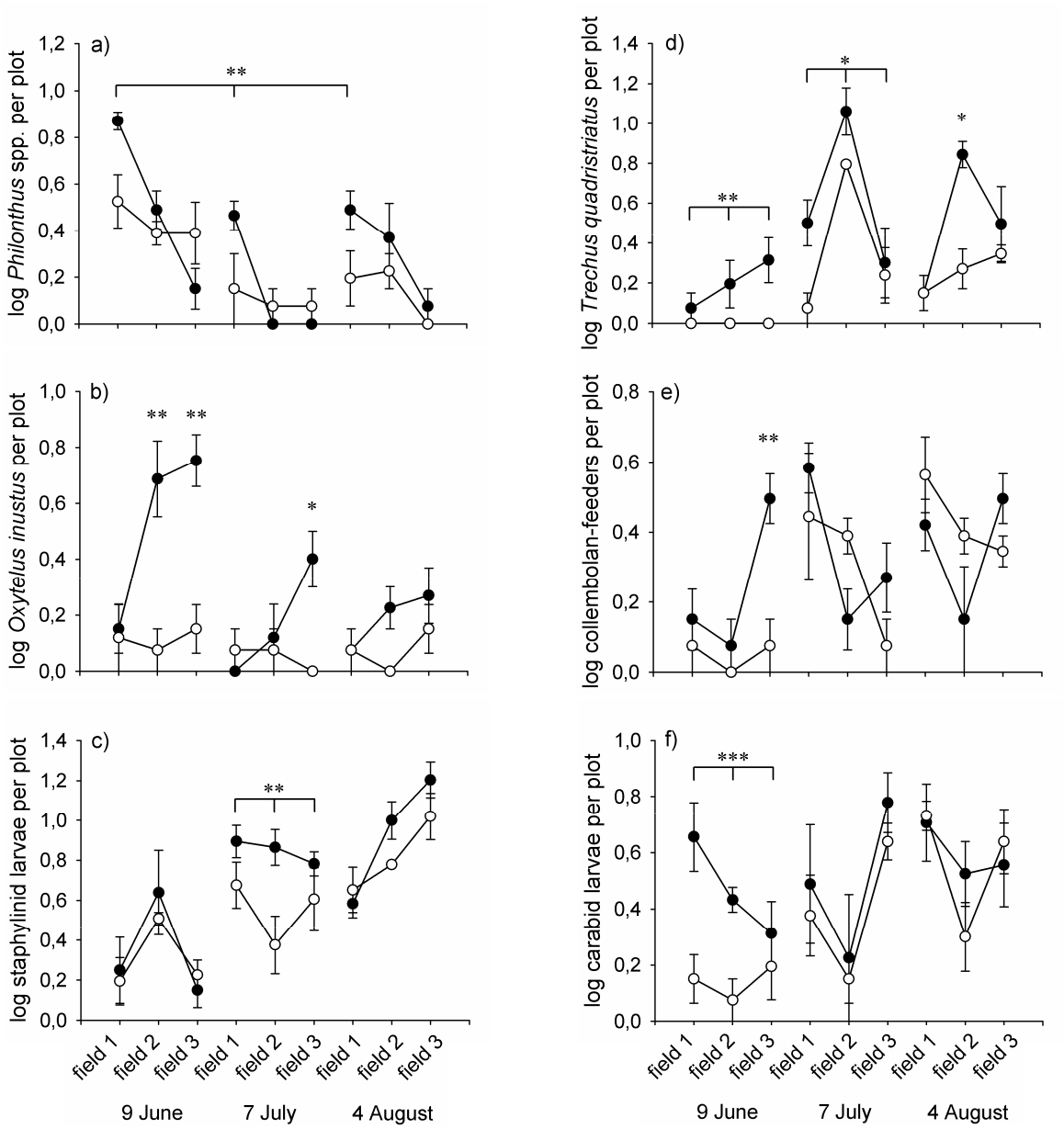

Fig. 1 Mean population densities (given as log numbers ± SE per 2 m^2) of a)-d), f) ground dwelling generalist predators, e) a collembolan feeder guild g)-i) epigeic collembolans in no-mulch -○- and mulch -●- treatments in the three different fields at three consecutive sampling dates (9 June, 7 July, 4 August). Significant differences between means are marked, lines connecting means indicate significant differences within sampling dates or fields (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; (*), $P < 0.1$).

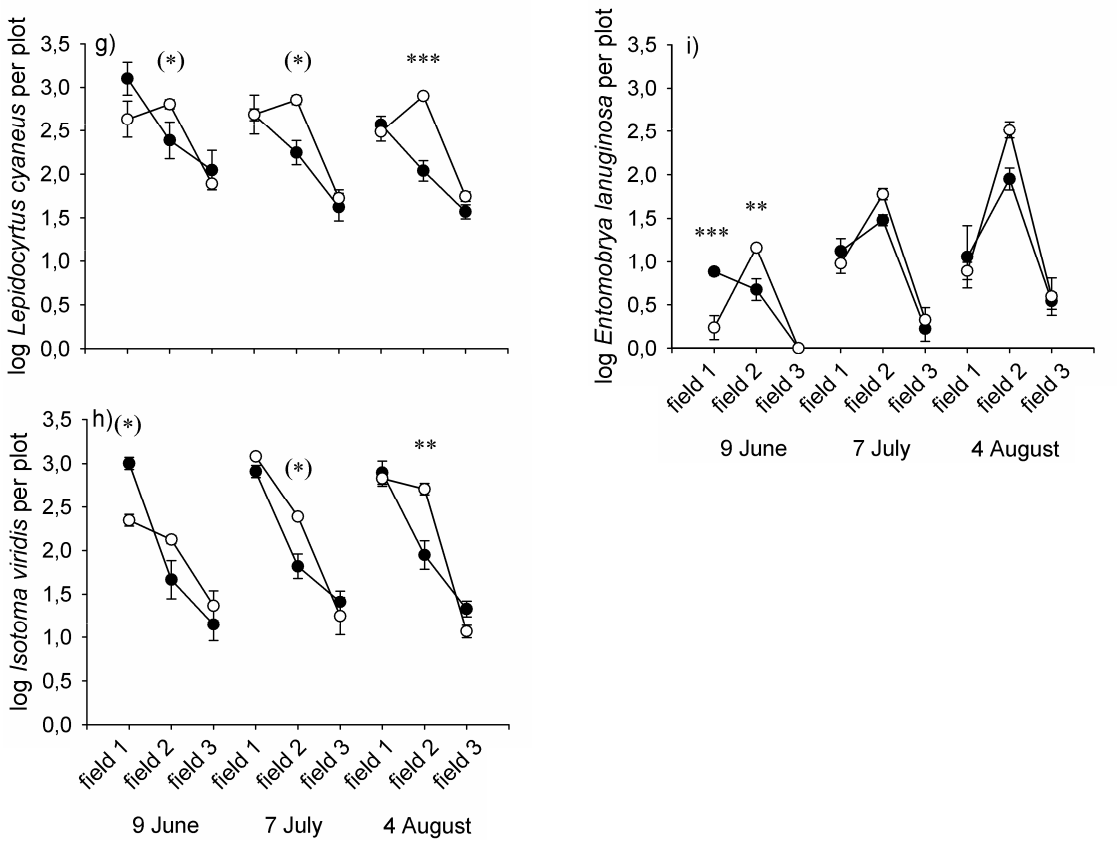

Fig. 1 extended

^{13}C and ^{15}N analysis

The $\delta^{15}N$ signatures of the 13 soil living species sampled spanned over two trophic levels in the mulch treatment (Fig. 2). The first trophic level comprised of decomposers such as the diplopods *Polydesmus inconstans*, *Brachyiulus pusillus* and *Blaniulus guttulatus* as well as the collembolan *Onychiurus* spp. and sciarid larvae. $\delta^{15}N$ signatures of decomposers formed a continuum from 1 ‰ to 3.1 ‰. The carabid species *Trechus quadristriatus* was also placed in the decomposer trophic level, with $\delta^{15}N$ signatures similar to the diplopod *Blaniulus guttulatus*. The upper trophic level consisted of predators such as the centipedes *Lithobius microps* and *Necrophloeophagus longicornis* and the gamasid mite *Pergamasus* sp., the Symphyla and the carabid *Oxytelus inustus*. Also, $\delta^{15}N$ signatures of elaterid larvae were similar to those of predators. In contrast, the sampled elaterid larvae in the no-mulch treatment had a $\delta^{15}N$ signature of only 2.9 ‰.

The incorporated maize-born carbon ranged between 15 % in the Symphyla and 65 % in the diplopod *Brachyiulus pusillus* (Fig. 2). No differences in the range of percentages of incorporated maize-born carbon were observed between the decomposer species (20 % to 65 %) and the predator species (15 % to 56 %).

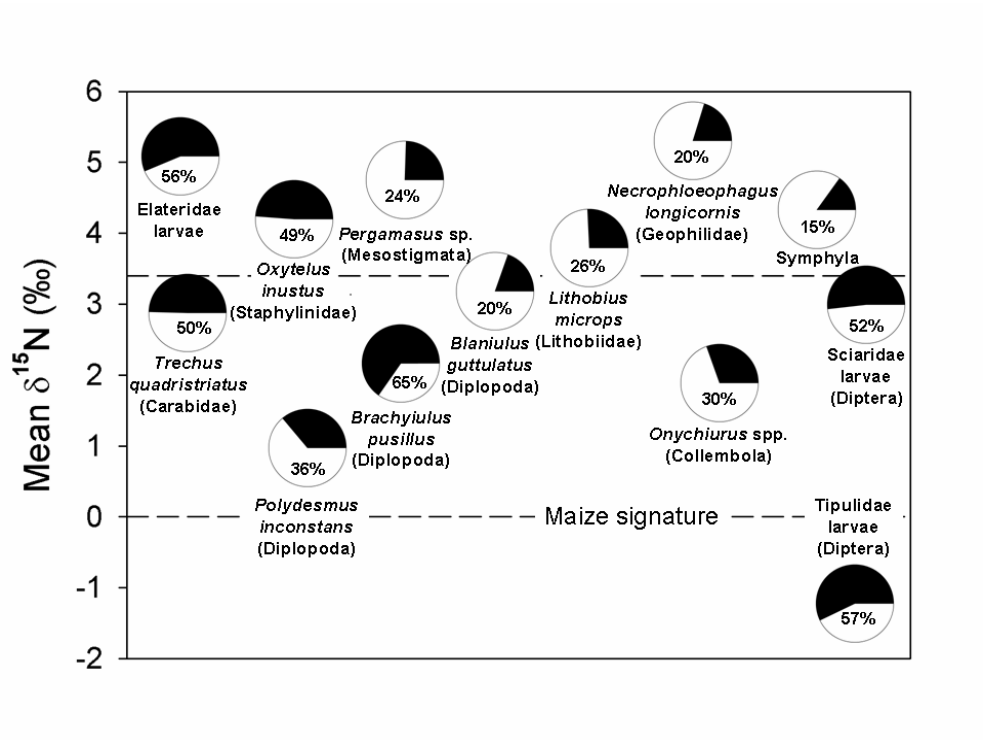

Fig. 2 Mean $\delta^{15}N$ values (‰) and maize carbon fraction of the body tissue carbon (%) of soil dwelling taxa.

The $\delta^{15}N$ signatures of all above-ground species studied spanned in the no-mulch and the mulch treatment over 7.2 ‰ and 6.7 ‰, respectively (Fig. 3a b). Assuming enrichment in ^{15}N of about 3 ‰ per trophic level (Minagawa & Wada 1984; Wada *et al.* 1991; Post 2002), the 18 species studied in the no-mulch and the mulch treatments spanned over three trophic levels. In the no-mulch treatment, the two collembolan species *Entomobrya lanuginosa* and *Isotoma viridis* had similar $\delta^{15}N$ signatures to the carabids *Trechus quadristriatus* and *Bembidion obtusum*. In the mulch treatment, $\delta^{15}N$ signatures of *Entomobrya lanuginosa* and *Isotoma viridis* were 1.1 ‰ and 1.4 ‰ lower compared to the no-mulch treatment (Fig. 3c), thereby building a distinct group with the collembolan *Lepidocyrtus cyaneus* between the two aphid species and the predators.

Only two of the 18 species above-ground significantly differed in their $\delta^{13}C$ signatures between the mulch and the no-mulch treatment. The body of the carabid *Trechus quadristriatus* and the staphylinid *Oxytelus inustus* contained 50 % and 49 % of maize-born carbon, respectively. Interestingly, only these two species were found in both, the pitfall traps and the soil cores.

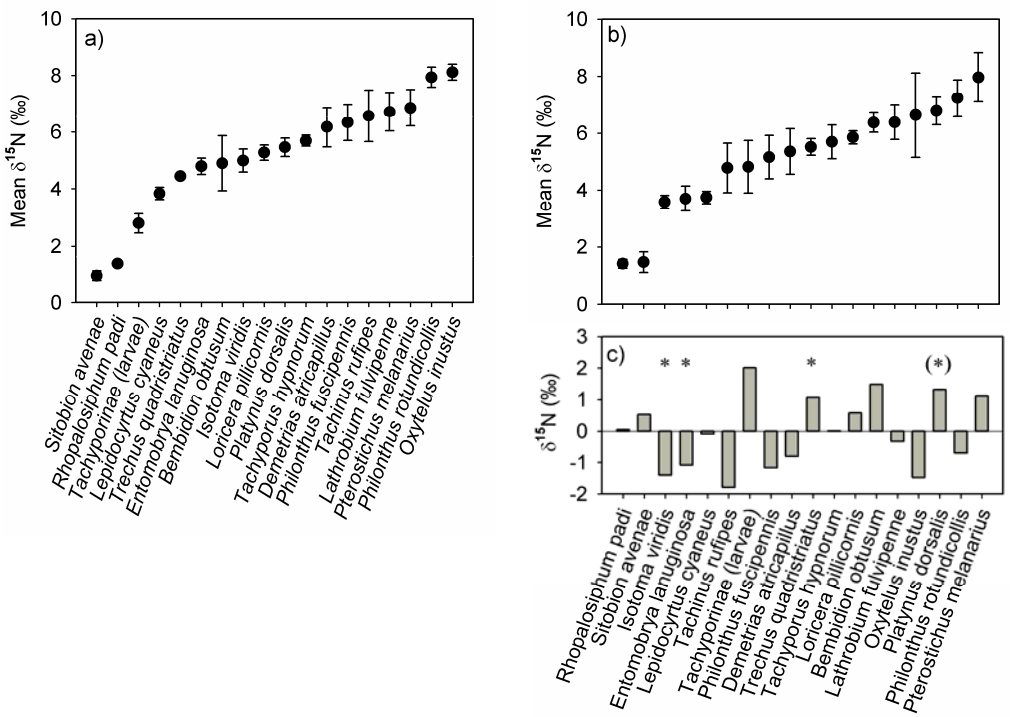

Fig. 3 Mean $\delta^{15}N$ values (‰) and SE of aboveground species in the a) no-mulch and b) mulch treatment and c) differences in $\delta^{15}N$ signatures of the aboveground species between the no-mulch and the mulch treatment. Significant differences between means are marked (*, $P < 0.05$; (*), $P < 0.1$).

Aphids

The dominant aphid species in all three fields was *Sitobion avenae*, representing 91.1 % of total aphid numbers, followed by *Rhopalosiphum padi* (6.3 %) and *Metopolophium dirhodum* (2.6 %). Aphid infestations averaged 19.8 individuals per shoot, being markedly above the threshold level of economic damage (five aphids per shoot, Giller *et al.* 1995). Aphid numbers on wheat plants growing in the pots and wheat plants growing in the field did not differ, nor were there any significant interactions between the factor 'pot' and any of the other factors (Table 1). Therefore, the factor 'pot' was excluded from the statistical model.

Total aphid numbers differed significantly between the three fields, with 6 fold and 22 fold higher aphid numbers in field 1 compared to fields 2 and 3, respectively ($F_{2,72} = 380.18$, $P < 0.001$). Aphid numbers also differed significantly between no-mulch and mulch plots in two of the three fields. In field 1 aphid numbers were 54 % higher in the no-mulch treatment compared to the mulch treatment whereas in field 3 aphid numbers were 77 % lower in the no-mulch treatment (Fig. 4).

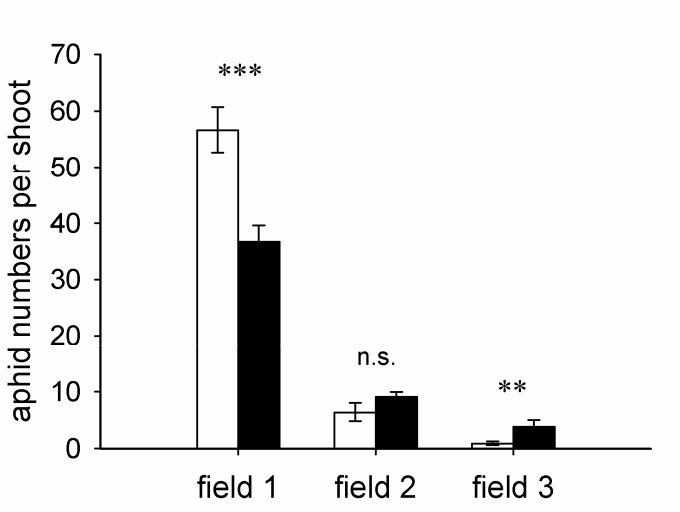

Fig. 4 Aphid populations (given as aphid numbers per shoot) in the no-mulch (open bars) and mulch (black bars) treatments in the three different fields. Significant differences between means are marked (**, $P < 0.01$; ***, $P < 0.001$, n.s. = not significant).

Table 1 ANOVA table of *F*-values for the effect of pot (yes/no), field (1, 2, 3), mulch (yes/no), ground dwelling predators (GP; reduced, control) and flying predators (FP; reduced, control) on aphid populations.

factor	d.f.	*F*	*P*-value
pot	1	0.95	0.336
field	2	294.65	< 0.001
mulch	1	0.01	0.925
GP	1	8.91	0.004
FP	1	7.87	0.007
pot*field	2	0.12	0.889
pot*mulch	1	1.11	0.298
field*mulch	2	16.25	< 0.001
pot*GP	1	0.15	0.703
field*GP	2	0.42	0.663
mulch*GP	1	3.26	0.077
pot*FP	1	0.34	0.564
field*FP	2	2.80	0.071
mulch*FP	1	0.04	0.841
GP*FP	1	0.32	0.573
pot*field*mulch	2	0.22	0.805
pot*field*GP	2	0.29	0.751
pot*mulch*GP	1	0.001	0.972
field*mulch*GP	2	0.47	0.630
pot*field*FP	2	1.03	0.364
pot*mulch*FP	1	0.15	0.699
field*mulch*FP	2	1.83	0.171
pot*GP*FP	1	0.41	0.523
field*GP*FP	2	3.36	0.043
mulch*GP*FP	1	4.29	0.044
pot*field*mulch*GP	2	0.12	0.884
pot*field*mulch*FP	2	0.14	0.868
pot*field*GP*FP	2	0.33	0.723
pot*mulch*GP*FP	1	0.001	0.971
field*mulch*GP*FP	2	0.59	0.560
pot*field*mulch*GP*FP	2	0.10	0.906

The effect of ground dwelling predators and flying predators differed significantly between the mulch and the no-mulch treatment (mulch × GP × FP: $F_{1,72} = 5.54$, $P = 0.021$). In the mulch plots, presence of ground dwelling predators and the combination of ground dwelling and flying predators significantly decreased aphid numbers by 45 % and 54 %, respectively, whereas there was no significant effect of flying predators alone on aphid populations (Fig. 5a). In the no-mulch plots, neither ground dwelling or flying predators alone nor their combination significantly affected aphid populations. Irrespective of mulch, predator effects differed significantly between fields (field × GP × FP: $F_{2,72} = 4.34$, $P = 0.017$; Fig. 5b). In field 1, aphid numbers were only decreased in plots with ground dwelling and flying predators (-34 %). In field 3, ground dwelling predators and the combination of ground dwelling and flying predators significantly decreased aphid numbers by 80 % and 85 %, respectively, whereas in field 2 there were no effects on aphid populations in any of the predator treatments.

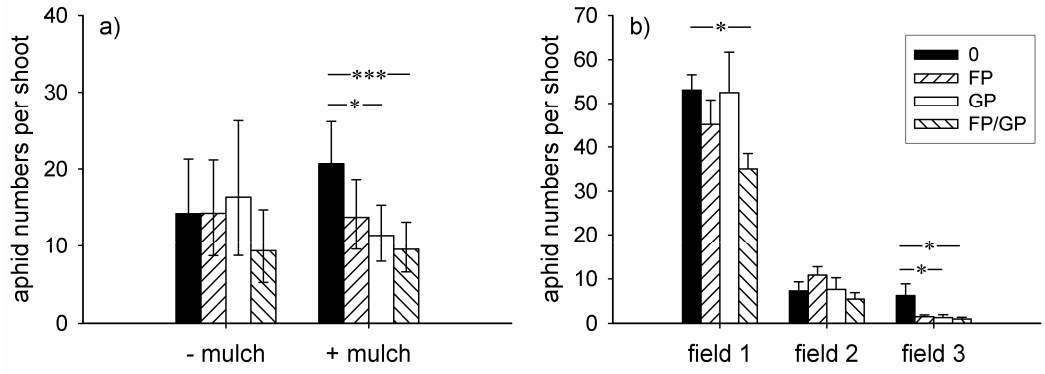

Fig. 5 Aphid populations (given as aphid numbers per shoot) as affected by flying (FP) and ground-dwelling (GP) predators in a) the no-mulch and the mulch treatments and b) in the three different fields. Error bars indicate standard error, significant differences between means are marked (*, $P < 0.05$; ***, $P < 0.001$).

2.5. DISCUSSION

Soil food web – maize-born carbon incorporation

About nine month after the application of the maize chaff, maize-born carbon could be detected in all taxa of the sampled soil fauna, indicating strong utilisation of the mulch material. The percentages of incorporated maize carbon in soil fauna species (excluding *Trechus quadristriatus* and *Oxytelus inustus*) varied between 15 % and 65 %, and differed between predators and decomposers. In decomposers the maize-born carbon contributed on average 43.3 % to animal tissue carbon, whereas predators on average contained 28.2 % maize-born carbon. This difference in incorporation of maize carbon between trophic groups is similar to that found in another agricultural soil food web (Albers *et al.* 2006), suggesting a time lag in carbon incorporation in higher trophic levels.

Assuming a trophic level shift in $\delta^{15}N$ signatures of 3 ‰ (Minagawa & Wada 1984; Post 2002), the soil food web consisted of three trophic levels. Soil food webs in agricultural fields investigated by Moore (1994) varied between 2.3 and 4.2 trophic levels, and Albers *et al.* (2006) also found three trophic levels in their agricultural soil food web. The $\delta^{15}N$ signatures of the species analysed formed a gradient rather than discrete trophic groups. However, the $\delta^{15}N$ signatures of the predators spanned only over 1.5 $\delta^{15}N$ units and did not differ between large and small predators, suggesting that predator species consisted of a single trophic group with little evidence for intraguild predation and cannibalism. In contrast, $\delta^{15}N$ signatures of decomposers spanned over 4.5 $\delta^{15}N$ units, suggesting that decomposers consist of two trophic levels, i.e. primary and secondary decomposers as indicated earlier (Scheu & Falca 2000).

Combining the $\delta^{15}N$ and $\delta^{13}C$ signatures, assumptions can be made about potential trophic links. With similar contents of maize carbon and about 2.5 $\delta^{15}N$ units higher than the collembolan *Onychiurus* spp., Symphyla, *Lithobius microps* and *Pergamasus* sp. presumably fed on this species; similarly, Albers *et al.* (2006) assumed Symphyla and Gamasina to prey on *Onychiurus* spp. As the $\delta^{15}N$ signature of elaterid larvae was similar to those of the predatory mite *Pergamasus* sp. and the centipede *Necrophloeophagus longicornis* they presumably live as predators; recent studies suggest that this is widespread in elaterid larvae

(Traugott *et al.* 2007). Interestingly, $\delta^{15}N$ signatures of the diplopod *Blaniulus guttulatus* exceeded those of the other two diplopod species, being similar to the $\delta^{15}N$ values of the centipede *Lithobius microps*. As suggested previously *Blaniulus guttulatus* presumably lives on animal diets, e.g. by feeding on carcasses (Hoffman & Payne 1969).

Aboveground food web – maize-born carbon incorporation

Only two of the sampled species in our study, the carabid *Trechus quadristriatus* and the staphylinid *Oxytelus inustus*, were abundant below- and aboveground as indicated by trapped individuals in both the soil cores and the pitfall traps. Interestingly, only these two species had incorporated maize-born carbon, whereas in none of the other 13 aboveground predator species maize-born carbon could be detected. Moreover, also in the three epigeic collembolan species, no maize-born carbon was found, suggesting that these species did not utilise the maize litter as a food source. C4 plants, such as maize, have been documented to be of low food quality; due to their low nitrogen and high fiber content nutritional parts of the plants are difficult to reach for herbivores and detritivores (Caswell & Reed 1976; Boutton *et al.* 1978; Ehleringer *et al.* 2001). Omnivory is probably the prevailing feeding strategy in collembolans (Filser 2002). Depending on the resources available they ingest bacteria, fungi, algae, plant litter, or other soil animals, such as protozoa, nematodes, rotifers, and enchytraeids (Parkinson 1988; Rusek 1998; Scheu 2002). Despite the fact that food resources of the three epigeic collembolans remain unknown, they were not linked trophically to the maize carbon pool.

Although maize-born carbon did not enter the majority of species of the aboveground food web, the maize mulch shifted $\delta^{15}N$ signatures of two collembolan species and of some predator species. The $\delta^{15}N$ signatures of *Isotoma viridis* and *Entomobrya lanuginosus* decreased significantly in the mulch plots, thereby grouping the three collembolan species between the two aphid herbivores and the predators. Presumably, the collembolan species changed their feeding to a more basal food source; unfortunately the nature of this source remains obscure. Some predator taxa showed similar decreases in their $\delta^{15}N$ signatures, but not significantly, presumably indicating that these predators mainly feed on collembolans. As all predator species in both the no-mulch (except for the Tachyporinae larvae) and the mulch treatments had $\delta^{15}N$ signatures more than 3 ‰ higher than those of the two aphid species, and

the most abundant prey species were collembolans and aphids, a mixed diet of these two prey taxa can be suggested for many of the generalist predators. Including collembolans and aphids in mixed diets improves development, survival and egg production of generalist predators (Toft 1995; Borg & Toft 1999; Bilde *et al.* 2000; Oelbermann & Scheu 2002), and complementary predation on aphids and collembolans was previously shown in a field experiment in winter wheat (Chapter 3). In contrast to the soil food web, intraguild predation and cannibalism possibly existed in the aboveground system, as $\delta^{15}N$ signatures of the predators spanned over 3.7 ‰ and 3.2 ‰ in the no-mulch and mulch treatments, respectively. Intraguild predation and cannibalism has been shown to be common in terrestrial food webs (Polis *et al.* 1989; Polis 1991); however, in soil food webs of arable systems it seems to be of minor importance as indicated by (Albers *et al.* 2006) and the present study.

Effects of mulch on predator densities – feedbacks on aphid suppression

Despite the abundance of 34 carabid beetle species, only one species (*Trechus quadristriatus*) was significantly increased in density in the mulch treatment. Furthermore, of the twelve sampled staphylinid genera, the density of only two species (*Philonthus fuscipennis* and *Oxytelus inustus*) was significantly increased in the mulch treatment. Except *Philonthus fuscipennis*, these species had incorporated maize-born carbon, suggesting that they in fact benefited from the maize resource presumably through feeding on decomposer prey. Additionally, the densities of carabid and staphylinid larvae were increased in the mulch treatment. However, the effect of mulch on these taxa as well as on the staphylinid *Philonthus fuscipennis* likely was indirect rather than through predation on decomposer prey as no maize-born carbon was detected in these taxa. Thomas *et al.* (2002) suggest that mobile species are able to respond rapidly to changing environmental conditions and food availability; and different soil textures likely influence oviposition, larval development and survival. Furthermore, enhanced prey availability has been shown to trigger predator aggregation (Niemela *et al.* 1986; Kielty *et al.* 1996; Bohan *et al.* 2000), and our mulch application presumably increased prey species, as maize carbon was used as additional resource by decomposer soil invertebrates. Therefore, enhanced densities in larvae of staphylinids and carabids in our mulch treatment indicate that these taxa had been attracted to these sites and

may have contributed to the observed reduction in aphid populations. Addition of mulch therefore may also enhance the control of pest species by attracting predators; once attracted they may contribute to pest control without benefiting from prey of the decomposer system. In fact, generalist predators in arable fields, such as carabids, are highly mobile and shift habitats from one generation to the other (Thomas *et al.* 1998).

Despite the apparently minor effect of maize mulch on predator densities, aphid numbers were significantly reduced by generalist predators in the mulch treatment. As only few predator species were affected by maize mulch, control of aphid populations was likely due to these predators, i.e. *Philonthus fuscipennis*, *Oxytelus inustus* and *Trechus quadristriatus*, as well as to carabid and staphylinid larvae. Interestingly, effects of maize mulch on densities of these predators differed significantly between fields. Moreover, only in two (field 1 and 3) of the three investigated fields, aphid populations were significantly decreased by generalist predators. By combining the data of field specific effects of mulch on predator densities and predator effects on aphid populations, single predator species can be identified as most effective control agents. With significant predator effects on aphid populations in mulch plots in field 1 and simultaneously increased densities of the staphylinid *Philonthus fuscipennis* exclusively in mulch plots in this field, *Philonthus fuscipennis* most likely contributed to the significant aphid suppression in this field. Using molecular gut content analyses, *Philonthus fuscipennis* has been shown to consume aphids at high rates (Chapter 6). Furthermore, this species has been demonstrated to effectively control aphid populations in previous field studies (Sopp & Wratten 1986; Dennis & Wratten 1991). In the other field (field 3) where ground dwelling predators effectively suppressed aphid populations in mulch plots (-80 %), densities of *Philonthus fuscipennis* where negligible. In this field densities of *Trechus quadristriatus* and *Oxytelus inustus* likely contributed to aphid control, as their densities were significantly increased in the mulch plots. *Trechus quadristriatus* is known to prey on aphids (Sunderland *et al.* 1987; Mundy *et al.* 2000) and *Oxytelus inustus*, a species which is very common in agricultural fields (Krooss & Schaefer 1998; Markgraf & Basedow 2002), has been shown to be carnivorous (Eghtedar 1970). Interestingly, densities of these two species were also increased in field 2, however no effects on aphid populations were observed. Failure in aphid suppression has been recorded in some field studies (Holland *et al.* 1996;

Holland & Thomas 1997a,b; Collins *et al.* 2002). Generally aphid control is assumed to be most effective early in the season (Edwards *et al.* 1979; Chiverton 1986) and densities of *Trechus quadristriatus* in field 2 were most increased at the second and third sampling date when aphid populations may have escaped predator control. The effect of carabid and staphylinid larvae is difficult to predict as they were not determined to species level and likely constitute of a number of species. Several carabid and staphylinid larvae have been documented to prey on aphids (Sunderland *et al.* 1987; Dennis *et al.* 1991; Theiss & Heimbach 1993; Kollat-Palenga & Basedow 2000; Kyneb & Toft 2004) and therefore the increased density of these larvae likely contributed to aphid suppression in our study.

Conclusions and prospects

The present study demonstrated the incorporation of detrital food resources (maize chaff) into an agricultural soil food web. Predator species occurring in both the above- and belowground system not only incorporated maize-born carbon but increased significantly in density in the mulch treatments. Effects of the added detritus propagated via generalist predators into the herbivore system, as predators significantly decreased aphid populations in mulch fields. Predator and herbivore densities as well as effects of mulch addition varied strongly between fields emphasising the necessity to investigate multitrophic interactions in a landscape context. Further, the results suggest that single generalist predator species can significantly contribute to herbivore suppression in agricultural systems, and these effects may be fostered by residue management practices. Knowledge of the factors driving the population dynamics of these species therefore allows to develop management practices which improve conservation biological control.

3 | Cereal aphid control by generalist predators in presence of belowground alternative prey: complementary predation as affected by prey density

3.1. ABSTRACT

Generalist predators are important antagonists of pest species in agroecosystems and potentially enhance conservation biological control. Increasing populations of alternative prey through detrital subsidies is one way to maintain those predators in fields. However, alternative prey may also distract generalist predators from their pest prey and thereby diminish the efficiency of biological control. To develop reliable predictions for biological control, it is essential to evaluate the relative importance of generalist predators, pests, alternative prey and their respective interactions. We investigated the effects of an assemblage of generalist predators on the grain aphid *Sitobion avenae* in winter wheat. Treatments with 10, 100 and 1000 aphids were established inside 2 m^2 sized caged plots. Three weeks after the experiment started, samples were taken to estimate the size of aphid populations and those of alternative prey that were sufficiently abundant. Three prey taxa were significantly reduced by generalist predators: the grain aphid *Sitobion avenae*, the click beetle *Adrastus pallens* and the springtail *Isotoma viridis*. Collembolans were decreased by generalist predators independent of aphid densities, indicating complementary predation of collembolans and aphids. At high aphid densities, grain aphid population peaks were decreased to the threshold level of economic damage, demonstrating efficient aphid suppression by the predator community. Click beetle numbers declined only at low and medium aphid densities. The results suggest that generalist predators preferentially fed on click beetles at low and medium aphid densities and switched to aphids at high aphid densities. Early-season predators likely had the greatest

influence on aphid suppression. Our results indicate that alternative prey from the belowground system forms a substantial food resource for generalist predators, suggesting that the belowground subsystem modulates predator-prey interactions above the ground.

3.2. INTRODUCTION

Generalist predators can be effective pest control agents as suggested by both biocontrol theory and practice (Symondson *et al.* 2002). They can decrease pest populations, thereby reducing plant damage and increasing plant yield (Östman *et al.* 2003). Therefore, developing management strategies enhancing biocontrol effects of generalist predators can be profitable for farmers. In agroecosystems generalist predators are confronted with a wide range of potential prey species beside the pest species. To better understand the processes that shape such interactions it is necessary to analyse generalist predators in a multitrophic and multispecies context. Predators may substitute or switch prey species as their relative abundances change (Holt & Lawton 1994); thus alternative prey (prey other than the target species) can distract generalist predators from feeding on the pest, i.e. disturbing biocontrol (van Baalen *et al.* 2001). However, alternative prey likely also contributes to maintain generalist predator numbers at times when pest is scarce, thereby enabling the predators to feed instantly on arriving pests (Symondson *et al.* 2002). The decomposer subsystem provides a food source for generalist predators to build up predator populations (Scheu 2001). Management strategies enhancing detritivores have shown to increase generalist predator numbers and are suggested to increase biocontrol effects (Settle *et al.* 1996; Halaj & Wise 2002). Collembola species proved to be of high food quality for generalist predators (Bilde *et al.* 2000) whereas cereal aphids have been shown to be of low food quality (Bilde & Toft 1994; Toft 1995), and this possibly interferes with biological control.

Aphids are the most important pests in cereal fields and can cause high damage and yield loss due to directly damaging the plants but also by transferring viral diseases (Vickerman & Wratten 1979). A number of studies have shown that generalist predators can suppress cereal aphids (Edwards *et al.* 1979; Chiverton 1986; Helenius 1990; Dennis & Wratten 1991; Lang

2003; Schmidt *et al.* 2003). However, there is evidence that aphid control is not always successful (Holland *et al.* 1996; Holland & Thomas 1997a,b; Collins *et al.* 2002), and failure in suppressing aphid populations has been ascribed in part to the presence of alternative prey (Dennis & Wratten 1991). Yet only a few field studies investigating the effects of generalist predators on cereal aphids have considered other abundant prey species (Lang *et al.* 1999; Östman 2004). Furthermore, no information is available on the role of alternative prey for generalist predators as affected by aphid density.

The present study investigates the effects of an assemblage of generalist predators on populations of the grain aphid *Sitobion avenae* F. in a winter wheat field. To evaluate possible prey density dependence of predatory effects, we established three different aphid densities in experimental plots whereas naturally abundant alternative prey was left unmanipulated. We hypothesized that (i) alternative prey including Collembola and other herbivores form a substantial part of the food of generalist predators; (ii) predators switch from alternative prey to aphids at increasing aphid densities and if the previous assumption is true, (iii) generalist predators are able to reduce aphids even at high aphid densities.

3.3. MATERIALS AND METHODS

The experiment was conducted in 2005 in an 8 ha winter wheat field managed by the Reinshof research farm of the University of Göttingen (Lower Saxony, Germany). No insecticides were applied in the year of the experiment. Experimental treatments were established in a 2 x 3 factorial design with factors ‘Generalist Predators (GP)’ (reduced and control) and ‘Initial Aphid Density Level (IADL)’ (low, medium and high). Each treatment was replicated four times resulting in 24 experimental plots. The plots were installed mid of May, with plastic barriers reaching 10 cm into soil and 40 cm aboveground enclosing a circular area of 2 m². Four ‘live’ pitfall traps (without trapping liquid) situated at the inner edge of the barriers were established in the predator reduced plots in a cross design. Pitfalls were cleared daily over a period of 3 weeks. All predators, i.e. carabids, staphylinids and lycosids, were visually identified, counted and released outside the plots. All other animals

were returned inside the plots. At dates when predators were captured in high numbers they were allocated to the control plots; each control plot received six additional predator individuals. This increased the field density only little and the resulting density of predators was well within the range occurring in the field. By adding the predators we intended to homogenise predator communities in the control plots rather than increasing their density. To inhibit aphid immigration into the plots, mosquito nets (mesh size 5 mm) were installed before aphid infestation in mid of June. Nets were cylindrical with the top closed, having the same diameter as the plastic barriers. They were supported by four plastic poles, buried 50 cm into the soil at the inner edge of each plot. On top of the four poles a ring of metal with the same diameter as the plastic barriers carried the net. Construction projected 150 cm above the ground providing enough space for wheat growth. In early July *Sitobion avenae*, reared in the laboratory, were transferred into experimental plots in batches of 10, 100 and 1000 individuals, forming the IADLs 'low', 'medium' and 'high'. To avoid any exchange of aphids and other animals between plots and surrounding area, Mosquito nets were additionally attached with pieces of wire to the plastic barriers. After 3 weeks, aphids were counted on 25 tillers per plot. To determine the surface active fauna, four pitfall traps were established in each of the plots as described above for live pitfall traps. Pitfall traps were filled with saltwater and operated for 2 weeks. Samples were stored at -10°C until determination. All predators as well as abundant potential prey were determined to species level.

Statistical analysis

Data on prey abundances were analysed by multivariate analysis of variance (MANOVA) with the dependent variables 'aphids', '*Adrastus pallens*', 'linyphiid spiders' and '*Isotoma viridis*'. Independent variables were 'Generalist Predators' (GP; reduced and control) and 'Initial Aphid Density Level' (IADL; low, medium, high). We used Pillai's trace as multivariate test since it is robust to deviations from multivariate normality (Quinn & Keough 2002). Subsequently, univariate ANOVAs (MANOVA-protected ANOVAs; Scheiner & Gurevitch 2001) were performed to evaluate the influence of the two independent variables on each single prey species. To test for the existence of differences in IADLs at the end of the experiment, we used one-way ANOVA for aphid numbers in the predator reduced treatment.

Where appropriate, Tukey´s HSD was used for multiple post-hoc comparisons. To improve homogeneity of variance data were $\log_{10}(x+1)$ transformed. In one of the experimental cages aphid densities were ca. 6 fold higher compared to the mean, showing a clear outlier counteracting our experimental setup. To standardise and secure statistically the procedure of identifying those cages, outliers were identified calculating the studentized deleted residuals (S. DRes.) for observations in each dependent variable. Observations with values of S. DRes > 2 or < -2 (i.e. observations with values more than 2 SDs from the regression line) were excluded from the analysis. Statistical analyses were calculated using Statistica 7.1 (StatSoft, Tulsa, USA).

3.4. RESULTS

In total 111 carabids, 350 staphylinids and 25 lycosids were removed from the predator reduced plots at the beginning of the experiment. About 94 % of the captured carabids and 43 % of total captured staphylinids were determined to genus or species level in the field. The most abundant carabids were *Bembidion* sp. (38 %) followed by carabid larvae (18 %) and *Platynus dorsalis* Pontoppidan (16 %) (Table 1). In staphylinids *Philonthus* sp. (60 %; mainly *Philonthus fuscipennis* Mannh.) was most abundant followed by *Tachyporus* sp. (23 %; mainly *Tachyporus hypnorum* L.) (Table 1).

At the end of the experiment numbers of early-season predators were considerably lower inside the control plots compared to the predator reduced plots at experimental setup. Numbers of *Philonthus fuscipennis* had decreased by 84.6 %, numbers of *Tachyporus hypnorum* by 66.7 % and numbers of *Platynus dorsalis* by 58.8 % (Table 1).

Table 1 Densities of predator and prey species per plot (2 m²) at the two sampling dates. (aboveground predators, n = 12 (20.5.-9.6. treatment –GP; 24.7.-7.8.; treatment +GP); Elateridae, Isotomidae and Lyniphiidae, n = 24 (24.7.-7.8.); arithmetic means ± SD.)

			20.5. - 9.6.	24.7. – 7.8.
Aboveground predators	Staphylinidae	*Philonthus fuscipennis*	7.58 ± 1.85	1.17 ± 1.07
		Tachyporus hypnorum	2.75 ± 1.36	0.92 ± 1.19
	Carabidae	*Bembidion lampros*	3.17 ± 2.15	0.08 ± 0.28
		Trechus quadristriatus	0.17 ± 0.37	7.42 ± 2.66
		Platynus dorsalis	1.42 ± 2.02	0.58 ± 0.86
		Pterostichus melanarius	1.08 ± 1.38	1.08 ± 1.04
Belowground prey	Elateridae	*Adrastus pallens*		16.48 ± 9.74
	Isotomidae	*Isotoma viridis*		154.55 ± 88.44
Aboveground prey	Linyphiidae			42.91 ± 11.06

In contrast, mid-season hatching predators were more abundant in the enclosures at the end of the experiment compared to experimental setup. The summer active carabid *Trechus quadristriatus* Schrank for example was 44.5 times more abundant at the second sampling date. In addition to aboveground predators three potential prey groups were highly abundant: the click beetle *Adrastus pallens* F., collembolans (mainly *Isotoma viridis* Bourlet) and linyphiids (Table 1).

Together with aphids, these four prey groups were tested for effects of the factors 'GP' and 'IADL'. Using Pillai's criterion the combined four dependent variables of potential prey organisms were significantly affected by both 'GP' ($F_{4,15} = 7.76$, $P = 0.001$) and 'IADL' ($F_{8.32} = 3.45$, $P = 0.005$) and the interaction between 'GP' and 'IADL' ($F_{8,32} = 2.36$, $P = 0.04$).

The effect of generalist predators on *S. avenae* numbers depended on IADL (Table 2). Predators significantly decreased aphid numbers by 62.7 % in the 'high' aphid density treatment, whereas there were no significant effects in the 'low' and 'medium' density treatments (Fig. 1a).

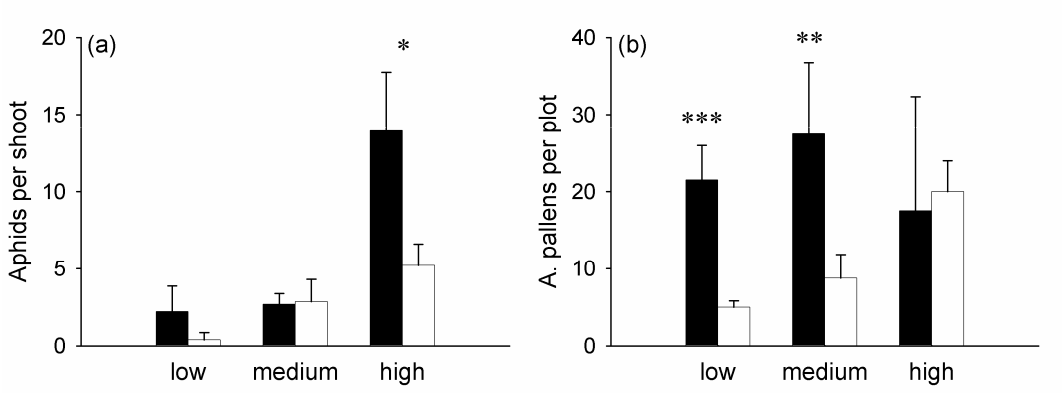

Fig. 1 a) population densities of aphids (given as aphid numbers per shoot) (a) and activity densities of *Adrastus pallens* given as *A. pallens* per plot (b) as affected by generalist predators at the end of the experiment (with generalist predators, open bars; with reduced generalist predator numbers, black bars) and initial aphid density levels ('low', 10 aphids; 'medium', 100 aphids; 'high', 1000 aphids). Significant differences between means are marked (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Activity density of *Adrastus pallens* significantly decreased in presence of generalist predators only in 'low' (-76.7 %) and 'medium' (-68.2 %), but not in 'high' aphid density treatments (Table 2 and Fig. 1b). Generalist predators significantly decreased activity density of *Isotoma viridis* by 52.3 % independent of aphid density (Table 2). Linyphiids generally did not respond to the factors 'GP' and 'IADL' (Table 2).

Table 2 Two factor ANOVA table of F-values for the effect of generalist predators (GP; reduced and control) and Initial Aphid Density Level IADL ('low', 'medium', 'high') on population densities (*Sitobion avenae*) and activity densities (*Adrastus pallens*, *Isotoma viridis*, lyniphiids) of prey taxa.

	GP	IADL	GP x IADL
Sitobion avenae	$F_{1,16} = 15.02$ $P = 0.001$	$F_{2,16} = 38.60$ $P < 0.001$	$F_{2,16} = 3.91$ $P = 0.041$
Adrastus pallens	$F_{1,15} = 22.05$ $P < 0.001$	$F_{2,15} = 3.35$ $P = 0.063$	$F_{2,15} = 10.02$ $P = 0.002$
Isotoma viridis	$F_{1,18} = 12.61$ $P = 0.002$	$F_{2,18} = 1.43$ $P = 0.264$	$F_{2,18} = 1.04$ $P = 0.374$
Lyniphiidae	$F_{1,16} = 1.67$ $P = 0.215$	$F_{2,16} = 0.26$ $P = 0.772$	$F_{2,16} = 0.91$ $P = 0.423$

At the end of the experiment, aphid densities in the predator reduced plots differed significantly ($F_{2,8} = 29.3$, $P < 0.001$); they were similar in the 'low' and 'medium' density treatment (2.2 ± 1.6 and 2.7 ± 0.7 individuals per shoot) and considerably higher in the 'high' density treatment (14.0 ± 3.8 individuals per shoot) (Fig. 2).

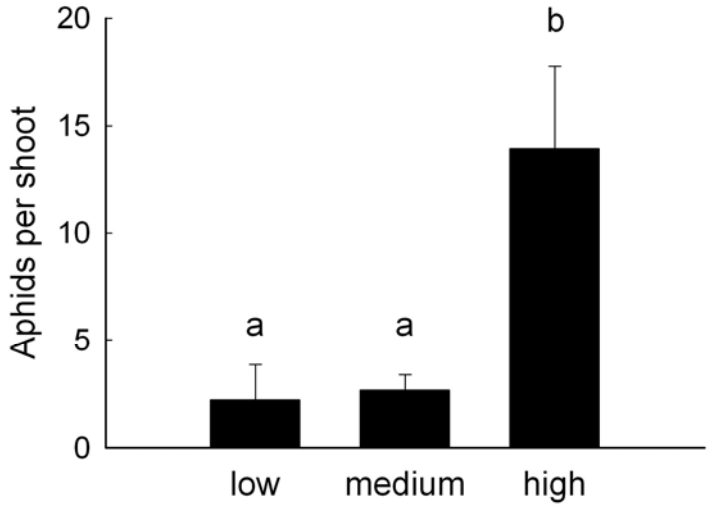

Fig. 2 Population densities of aphids (given as aphid numbers per shoot) as affected by the initial aphid density treatment in reduced predator numbers plots 'GP-' at the end of the experiment. Letters indicate significant differences between groups (Tukey's HSD, $P < 0.05$).

3.5. DISCUSSION

Manipulating the density of grain aphids using a standardised experimental approach permitted new insights in multipredator, multispecies interactions in an agroecosystem, including indirect effects of belowground prey on aphid control (Fig. 3). Three prey taxa, the grain aphid *Sitobion avenae*, the click beetle *Adrastus pallens* and the springtail *Isotoma viridis*, were significantly reduced in plots with ground dwelling generalist predators. The most abundant generalist predators *Bembidion*/*Trechus* spp. and *Platynus dorsalis* in carabids and *Philonthus fuscipennis* and *Tachyporus* sp. in staphylinids likely were responsible for this reduction. These species are polyphagous (Sunderland 2002) and are known to feed on aphids (Sunderland 1975; Sopp & Chiverton 1987), collembolans and other beetles (Sunderland 1975).

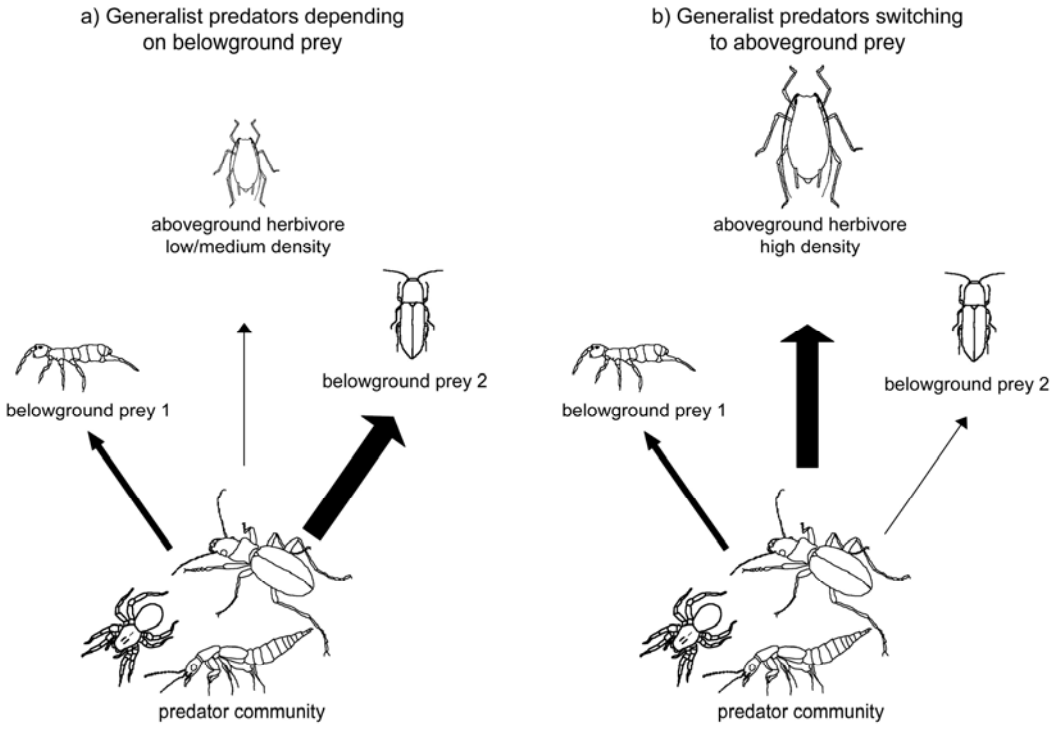

Fig. 3 Conceptual model showing the direct effects of a predator community (carabid beetles, staphylinid beetles, spiders) on aphids and two belowground prey species (litter-dwelling Collembola and click beetles, whose larval stage is belowground). We compare a) low to medium (10-100 individuals/m²) with b) high (1000 individuals/m²) aphid densities causing predators to switch prey. Thickness of arrows indicates relative population decrease of prey species as affected by generalist predators.

Two of the prey species, the grain aphid *Sitobion avenae* and the click beetle *Adrastus pallens*, were differently affected in the three aphid density treatments by the predator exclusion. Significant reductions of these two prey species were mutually exclusive, suggesting that predators preferentially fed on *Adrastus pallens* (presumably on currently hatched adults) at low aphid densities and switched to aphids when these became larger in numbers. Concordantly, aphids have been shown to distract predators from Colorado potato beetle eggs (Koss & Snyder 2005) as well as fly eggs (Prasad & Snyder 2006b) when becoming more abundant. Such positive prey-prey interactions where the presence of one prey species distracts the predator from the second prey (Holt 1977) can disrupt biological control if the second prey is the target prey (Settle & Wilson 1990). On the other hand, as shown in this study, such interactions can enhance switching from the alternative prey to the pest prey resulting in a dampening of pest population peaks.

In contrast to *Adrastus pallens*, Collembola were decreased in the generalist predator treatments irrespective of aphid densities, suggesting that the fraction of Collembola in the diet of generalist predators was independent of aphid density. Moreover, Collembola seemed to form a considerable amount of prey for generalist predators even at high aphid densities. The most abundant Collembola species at our study site was *Isotoma viridis*. This species is partly active on the soil surface and therefore highly available as prey for generalist predators. Indeed, a closely related species, *Isotoma anglicana*, proved to be preferential prey for generalist predators (Marcussen *et al.* 1999; Bilde *et al.* 2000) and including Collembola in their diet beneficially affected these predators (Bilde *et al.* 2000). In contrast, aphids are of comparatively low food quality for polyphagous predators (Bilde & Toft 1994; Jorgensen & Toft 1997a,b; Toft 1997), but including aphids in a mixed diet improves development, survival and egg production of generalist predators (Toft 1995; Borg & Toft 1999; Oelbermann & Scheu 2002). Reduced Collembola densities in the high aphid density treatment suggests that generalist predators indeed fed on both Collembola and aphids since aphid densities were also reduced.

Lyniphiid spiders were not affected by generalist predators. Lycosids as well as carabids have been shown to feed on and decrease densities of lyniphiids (Dinter 1998; Denno *et al.*

2004). Presumably only a small fraction of lyniphiids were captured with Pitfall traps, therefore assumptions on predatory effects are difficult.

Generalist predators significantly suppressed aphid populations in the high aphid density treatments. In predator reduced plots aphid numbers were increased by 180 % compared to the control. In most previous studies on the influence of generalist predators on *Sitobion avenae* aphid populations were only reduced at lower aphid densities (Edwards *et al.* 1979; Lang 2003; Schmidt *et al.* 2003), and failure to control aphids is usually ascribed to high aphid numbers and rapid growth rates (Holland *et al.* 1996; Holland & Thomas 1997b; Collins *et al.* 2002). In contrast, our results showed successful aphid suppression at high aphid densities. Furthermore, a rapid population growth of aphids in our cages most likely was due to the use of late instar apterae at experimental setup as these produce more offspring than alatae and early instar individuals (Wratten 1977; Dixon 1998). Indeed, aphid populations increased 10 fold during three weeks. Predation reduced aphid population growth from 10 fold to only 3.6 fold, keeping aphid numbers at the threshold of economic damage (3-5 aphids per tiller, Holz *et al.* 1994; Giller *et al.* 1995). Generally, the potential of generalist predators for controlling aphids is assumed to be strongest early in the year before aphid populations start growing exponentially (Edwards *et al.* 1979; Chiverton 1986; Burn 1992). Our results suggest that the identity of the predators abundant early in the year is more important for successful aphid control than the rate of aphid population growth. With early establishment of the predator exclusion barriers, we prevented predators to immigrate into the plots thereby conserving the early predator community. Early generalist predators, such as *Platynus dorsalis*, *Bembidion lampros* Herbst, *Philonthus fuscipennis* and *Tachyporus hypnorum*, are known to be most effective in reducing aphid numbers (Edwards *et al.* 1979; Sunderland *et al.* 1987). Indeed, these species were highly abundant in our plots, suggesting that these predators mainly contributed to aphid control. Especially *Philonthus fuscipennis*, the most abundant species in our plots, is known to effectively control aphids (Sopp & Wratten 1986). In a field experiment *Philonthus fuscipennis* decreased aphid numbers from 25 to seven individuals per shoot (Dennis & Wratten 1991) corresponding to our results.

At the end of the experiment the density of predators had converged in predator reduced and control treatments which presumably was due to the fact that (i) the predators added were

captured only in part at the end of the experiment and (ii) predators hatched from pupae in soil. In particular generalist predators active in mid-season, such as *Trechus quadristriatus* and *Pterostichus melanarius* Illiger, likely emerged from soil after establishment of the plots. In fact, low numbers of early active beetles and high numbers of mid-season active beetles were caught at the end of the experiment. Nevertheless, as indicated by significant changes in prey density our treatments must have been successfully established. Furthermore, high numbers of generalist predators in the predator reduced treatment at the end of the experiment suggests that also in this treatment prey density has been reduced by generalist predators. Therefore, the effect of generalist predators on the prey population in fact was more pronounced than we concluded from comparing predator reduced and control plots.

Effects on prey organisms by other predators than the generalists were unlikely since with the early caging of our plots we also excluded immigration of specialist predators such as parasitoids, syrphids or lacewings, which are known to control grain aphids (Schmidt *et al.* 2004; Thies *et al.* 2005). Supporting that these treatments were effective, no specialists or mummified aphids were found inside the experimental plots at the end of the experiment.

Conclusions

Complementary predation of above- and belowground prey appears to be an important predator-prey interaction affecting aphid suppression by generalist predators. This mechanism did not disrupt aphid control but possibly enhanced aphid predation. Moreover, even at high aphid densities predators suppressed aphid populations to the threshold level of economic damage. Presence of alternative prey enabled generalist predators to switch from belowground click beetles to aphids at increasing aphid numbers, leading to efficient aphid suppression. Collembola functioned as alternative food source for the predators independent of aphid densities, but did not distract predators from their aphid prey. The results suggest that alternative prey plays an important role for cereal aphid control as it contributes to high predator population densities without interfering with pest suppression. Hence, pest control in arable systems may be fostered by management practices enhancing alternative prey, e.g. via organic farming.

4 | Impact of abiotic factors on predator-prey interactions: DNA-based gut content analysis in a microcosm experiment

4.1. ABSTRACT

The effects of predators on prey populations can be modified by a number of abiotic factors. Here we investigated the combined and separate effects of rain and ground-dwelling predators on aphid populations in a microcosm experiment lasting for 21 days, using PCR to analyse the gut content of the predators. Rain significantly dislodged aphids from shoots and ears by 57 % and 25 %, respectively. The gut content analysis showed that more predators consumed aphids in the rain treatment than without rain, indicating higher availability of aphids for ground-dwelling predators after rain. However, no synergistic effects of rain and ground-dwelling predators on aphid population development could be demonstrated. Rain alone significantly decreased aphid populations by 27 %, suggesting that this is a significant mortality factor. Predators alone had no significant effect on aphid numbers, but the gut content analyses showed aphid consumption also in the no-rain treatments, indicating that aphids were available to the predators on the soil surface even without rain. Our results suggest that weather conditions such as wind and rain can modify predator-prey interactions in the field. Employing PCR-based predator gut content analyses proved to be useful as trophic links could be directly verified.

4.2. INTRODUCTION

Animal numbers are driven by interactions between abiotic and biotic factors, such as competition and predation (Krebs 2001; Begon *et al.* 2005). These factors do not work independently, rather they interact to affect population dynamics. It has been suggested that abiotic conditions can modulate predatory impacts on herbivores (Chase 1996; Stiling & Rossi 1997; Fraser 1998; Fraser & Grime 1998). Weather conditions, especially rainfall, have been proven to be a major factor regulating arthropod populations (Watson & Carter 1983; Masters *et al.* 1998; Frampton *et al.* 2000; Ovadia & Schmitz 2004). For example, rain dislodges aphids from plants or initiates inter-plant movement (Dhaliwal & Singh 1975; Zuniga 1991; Mann *et al.* 1995; Narayandas & Alyokhin 2006). A higher proportion of living aphids on the soil surface increases the potential of ground dwelling predators to control aphid numbers (Griffiths *et al.* 1985; Sopp *et al.* 1987; Losey & Denno 1998b). Several studies have demonstrated regulation of cereal aphids by ground-dwelling predators (reviewed in Symondson *et al.* 2002). Although within many of these studies the potential of abiotic effects, such as wind and rain, to affect predator-prey interactions have been discussed (Sunderland & Vickerman 1980; Dennis & Sotherton 1994; Holland & Thomas 1997a,b; Sunderland *et al.* 1997), we know of no previous studies that have specifically investigated the effects of interactions between rainfall and ground-dwelling predators on herbivores. Without assessing how abiotic factors shape predator-prey interactions the efficiency of herbivore control in the field is difficult to predict.

One reason for the lack of studies investigating how abiotic factors modify predator prey-interactions is the difficulty in evaluating trophic links, especially where predators are small, nocturnal or subterranean. Even in well controlled systems, such as microcosms, it is impossible to follow predator–prey interactions for extended periods. New techniques, particularly gut-content analysis using PCR and prey-specific primers, may allow unprecedented progress in quantifying who is feeding on whom without disturbing the system prior to predator collection (Symondson 2002; Sheppard & Harwood 2005). Recently this approach has been developed to study predator-prey links specific to agricultural systems (Agusti *et al.* 2003; Harper *et al.* 2005; Greenstone *et al.* 2007; Juen & Traugott 2007). The

ability to determine which prey species actually have been consumed by a predator allows opening the "black box" of trophic links of terrestrial systems. Surprisingly, no microcosm studies employing these promising molecular approaches has yet been reported.

Here we employed a PCR-based gut content analysis in a microcosm experiment investigating the effects of rainfall on predator-prey interactions by (a) assessing the immediate effect of rain on aphid consumption by carabid beetles (*Pterostichus melanarius*) and (b) determining the longer-term effects of rainfall and predation by *Pterostichus melanarius* on aphid population growth. We hypothesized that (i) rainfall dislodges aphids from wheat plants, making them more accessible to ground-dwelling predators, (ii) more predators will be able to consume aphids directly after rainfall compared to predators in no-rain treatments and (iii) synergistic effects exist between rainfall and ground-dwelling predators due to higher availability and consumption of prey after rain.

4.3. MATERIALS AND METHODS

Adult *Pterostichus melanarius* were collected by pitfall trapping from a winter wheat field near Darmstadt, Germany, during May and June 2006. Beetles were transferred individually into plastic containers (diameter 9.5 cm; height 4.5 cm) filled with damp potting compost and maintained in a controlled environment (16 °C; L:D 16:8) until the start of the experiment. Twice a week one larva of *Calliphora vomitoria* was fed to each beetle to ensure the same nutritional status of the beetles. Prior to the experiment the beetles were starved for five days. A polyclonal population of the aphid *Sitobion avenae* was cultured in glass containers on winter wheat at 24°C and L:D 16:8. Wheat plants were replaced regularly to keep aphid populations at low densities, to avoid development of alatae. To ensure similar reproduction rates between individuals, only late instar aphids were used in the experiments.

Experiments were conducted in a ventilated greenhouse in July 2006. Experimental treatments were established in a 2 × 2 factorial design with the factors 'Rain' (yes/no) and 'Predators' (yes/no). Each treatment was replicated 16 times resulting in 64 experimental pots. Microcosms (diameter 25 cm, height 25 cm) were filled to three-quarters with potting

compost covered with a 5 cm thick layer of field soil. The latter was taken from a ploughed crop field near Darmstadt in May 2006, sieved and heated to 60 °C for 3 h prior to the experiment to kill soil-living invertebrates. The upper layer of field soil was intended to simulate the soil surface structure of an arable field.

Each microcosm was planted with ten wheat plants, two plants in the middle and eight plants in an outer circle. Before wheat ear development each microcosm received approximately 50 aphids and three adult *Pterostichus melanarius*. To prevent aphids as well as predators from emigration or immigration from outside the microcosms, mosquito nets (mesh size 1 mm, tightly sealed with clips and tape) covered the microcosms up to 75 cm in height. Additionally, the smooth inner surface of the microcosm walls prevented beetles from escape. During the three-week experimental period, rain treatment microcosms were sprinkled with tap water (1 mm min^{-1}) for 5 min once a week, simulating a typical summer rain shower. All other microcosms received the same amount of water (0.25 l) directly on the soil surface. In the second week, 24 h after sprinkling the microcosms, one *Pterostichus melanarius* per microcosm was collected and frozen at -24 °C for subsequent gut content analysis. Three weeks after the start of the experiments the nets were carefully removed and the aphids in the microcosms were counted.

To evaluate the direct effects of the rain treatment on aphid dropping, four microcosms identical to the ones described above, were sprinkled for 1 min. Shortly before and immediately after sprinkling aphids on ears and shoots were counted.

DNA extraction and PCR

For DNA extraction each beetle gut was removed and homogenised in 50 µL of PCR water. For each beetle separate gloves were used to avoid sample-to-sample contamination. Twenty-five µL of the homogenate were utilised for DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers instructions. The DNA was resuspended in 200 µL of manufacturer's elution buffer and stored at -24 °C.

Aphid-specific primers S103 and A103 (Chapter 6) were used to amplify a 231 bp sized fragment of the mitochondrial cytochrome oxidase I gene (COI) of *Sitobion avenae*. PCRs were performed in 10 µL reactions containing 3 µL of extracted DNA, 0.25 mM dNTPs

(Fermentas), 1 µM of each primer, 1 µL 10 x buffer (Invitrogen), 3 mM $MgCl_2$, 0.12 µg bovine serum albumin (BSA, 10 mg/ml) and 1.5 U *Taq* DNA polymerase (Invitrogen). The DNA was amplified in an Eppendorf Mastercycler Gradient PCR machine, cycling conditions were 2 min at 94 °C, 40 cycles of 15 sec at 94 °C, 30 sec at 61 °C, 45 sec at 72 °C, and a final elongation step of 2 min at 72 °C. PCR water as well as DNA from *Pterostichus melanarius* and *Sitobion avenae* were included within each PCR to test for DNA carry-over contamination, false-negative and false-positive amplifications. PCR products were visualised on a 1.5 % agarose gel stained with ethidium bromide.

Statistical analysis

Data on aphid numbers were analysed by two-factorial analysis of variance (ANOVA) with the independent variables generalist predators (yes and no) and rain (yes and no). To improve homogeneity of variance data were $\log_{10}(x)$ transformed. The direct effects of rain on aphid dropping were analysed by a paired t-test comparing aphid numbers before and after raining on shoots and ears. To test for differences in aphid dropping rates between wheat-shoots and wheat-ears, the percent decrease of aphid numbers after rain was calculated for shoots and ears, respectively. Data were arcsine transformed and compared by paired t-test. Molecular data were analysed using a chi-square test to test for differences in the rates of beetles testing positive for aphid DNA between the 'rain yes' and the 'rain no' treatments. Statistical analyses were calculated using STATISTICA 7.1 (StatSoft, Tulsa, USA).

4.4. RESULTS

Aphid numbers (means ± s.e.) were significantly reduced in the 'rain yes' treatment from 3394 ± 290 to 2483 ± 166 individuals per microcosm ($F_{1,53} = 6.40$; $P = 0.01$) (Fig. 1). The presence of *Pterostichus melanarius* also reduced aphid numbers from 3119 ± 237 to 2795 ± 258 individuals per microcosm, but this decrease was not significant ($F_{1,53} = 2.55$; $P = 0.12$) (Fig. 1).

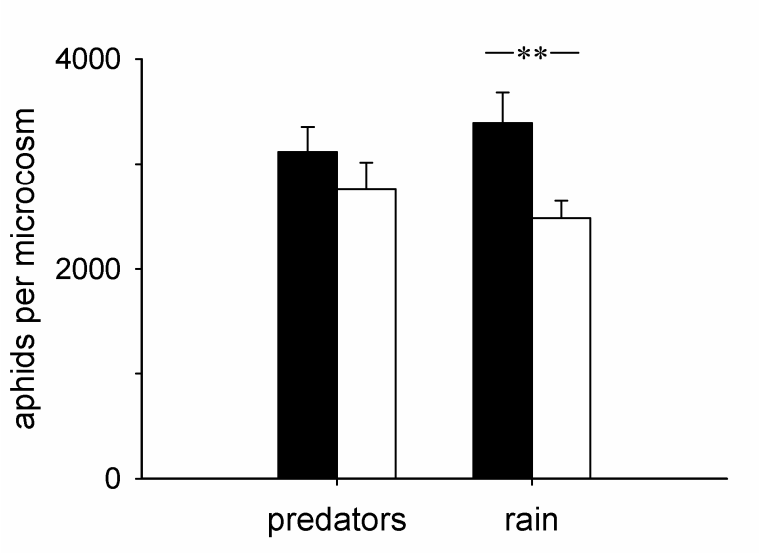

Fig. 1 Numbers of aphids (aphids per microcosm) as affected by ground dwelling predators (without predators black bars, with predators grey bars) and rain (without rain black bars, with rain grey bars;). Significant differences between means are marked (**, $P < 0.01$). Error bars are ± SE.

There was no significant interaction between rain and generalist predators. In the four microcosms used to determine the immediate effect of raining, aphid numbers decreased significantly after raining on ears and shoots from 140 ± 8 to 105 ± 11 and 81 ± 28 to 56 ± 12 individuals per microcosm, respectively (ears $t = 3.90$, $P = 0.03$; shoots $t = 26.34$, $P < 0.001$; paired t-tests) (Fig. 2). Numbers of aphids dropping from ears and shoots differed significantly ($t = -5.67$; $P = 0.01$; paired t-test) with ~32 % more aphids dropping from shoots than from ears (Fig. 2).

In the 'rain yes' treatment 69 % of the analysed beetles tested positive for aphid DNA compared to only 31 % in the 'rain no' treatment ($\chi^2 = 4.50$; $P = 0.03$).

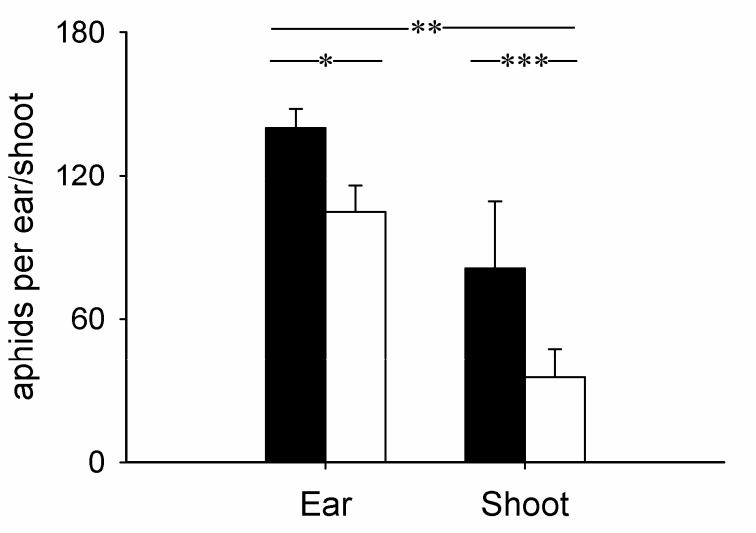

Fig. 2 Numbers of aphids per ears and shoots before (black bars) and after rain (grey bars). Significant differences between means are marked (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Error bars are ± SE

4.5. DISCUSSION

We investigated the combined and separate effects of biotic (ground-dwelling predators) and abiotic factors (rain) on aphid population dynamics on wheat. Combining an experimental microcosm approach with molecular techniques allowed determining directly how trophic interactions between ground beetles and aphids were affected by abiotic factors.

Rain significantly dislodged aphids from the wheat plants. On average more than 40 % of the aphids were displaced from ears and shoots after rain. Therefore, a high proportion of aphids was available on the soil surface as prey for *Pterostichus melanarius*. In fact, molecular gut content analysis identified more beetles containing aphid DNA in their guts in the rain treatment (69 %) than without rain (31 %). As the beetles for DNA analysis were sampled 24 h after the application of rain, this suggests that beetles consumed aphids which had been falling onto the soil surface. However, aphid populations in the rain treatments were not further reduced by *Pterostichus melanarius*; there was no significant reduction in aphid numbers by ground dwelling predators. DNA gut content analysis cannot distinguish between consumption of living prey by active predation and consumption of dead prey by scavenging (Foltan *et al.* 2005; Juen & Traugott 2005). Moreover, the PCR-based gut content analysis applied in the present study was qualitative and did not allow us to determine how many aphids each predator had consumed. Therefore, both types of prey (live and dead) could have contributed to the high detection rates in the beetles and indeed both types of prey were accessible for beetles. In fact carabid beetles consume both living and dead aphid prey if available (Chapter 5).

Even 24 h after rain was applied some of the dislodged aphids were active at the soil surface and therefore available as prey for *Pterostichus melanarius*. The time aphids survive off plants can exceed 24 h, as has been shown in laboratory experiments with the aphids *Acyrthosiphon pisum* and *Acyrthosiphon kondoi* (Losey & Denno 1998a). The ability of aphids to survive under wet conditions can be remarkable; flooded *Rhopalosiphum padi* survived at a rate of 98 % if they floated and 82 % if they became submerged (Araya & Fereres 1991). However, high numbers of aphids dislodged from the wheat plants by rain must have died at the soil surface as rain significantly decreased aphid populations by 27 %,

indicating that rain is an important mortality factor for aphids. Mann *et al.* (1995) suggested the mortality due to rain in *S. avenae* on wheat to be about 25 %. Dhaliwal and Singh (1975) reported 74 % mortality in the wheat aphid *Macrosiphum miscanthi* due to dislodgement by rain. Therefore, after rain, dead aphids are likely to form part of the diet for generalist predators such as *Pterostichus melanarius*. Presumably, *Pterostichus melanarius* consumed both living and dead aphids but at different ratios. This carabid is known to scavenge and has been shown to prefer fresh dead aphids over living ones (Foltan *et al.* 2005). In our experiment high numbers of dead aphids may have distracted *Pterostichus melanarius* from living aphid prey, and therefore no synergistic effect of rain and *Pterostichus melanarius* on aphid populations occurred. In fact, without having access to dead aphid prey in the no rain treatment aphid populations decreased by 12 % in the presence of *Pterostichus melanarius* (although this reduction was not significant). The detection of aphid DNA in beetle guts within this treatment suggests that aphids had been falling to the ground without the influence of rain, as *Pterostichus melanarius* was thought to be unable to climb the wheat plants by Griffiths *et al.* (1985). Predation of these aphids would not have been detectable without a DNA-based technique as the effect on aphid populations was not significant and would have been ignored.

Winder *et al.* (1994) estimated aphid availability for ground dwelling predators and suggested that aphid consumption in the field may often be limited simply by aphid availability. The authors concluded that total consumption would increase if aphid numbers increased. In fact, in our study rain increased aphid numbers on the soil surface causing higher predation rates. Predators which do not scavenge or have a strong preference for live prey, such as linyphiid (Fraser 1982; Sunderland *et al.* 1987) and lycosid spiders (Chapter 5), may contribute to synergistic effects caused by rain.

Adult aphids have been shown to have a higher risk falling off plants than younger nymphs (Dewar *et al.* 1982; Watson 1983; Cannon 1984). Furthermore, more aphids are dislodged from shoots than from ears. Similarly, in our microcosms, the proportion of dislodged aphids from shoots (57 %) was more than twice the number dislodged from ears (25 %). Also, Dhaliwal and Singh (1975) found a higher dislodgement of aphids from wheat plants without ears than from those with ears. Sopp *et al.* (1987) suggested that the peak of predation on

aphids by generalist predators in cereals, before wheat earing early in the season, is due to high numbers of aphids active on the soil surface. Rain probably contributes to dropping of aphids early in the year, triggering positive synergistic effects leading to high predation rates by generalist predators. Indeed, rain before the end of wheat flowering may prevent aphid outbreaks in cereal fields (Watson & Carter 1983), but interactions between dropped aphids and ground dwelling predators were not investigated in their study.

In conclusion, by applying artificial rain to a plant-herbivore-predator system, we demonstrated negative effects of rain on aphid population development. Moreover, by employing a DNA based gut content analysis, we showed for the first time that rain can affect insect predator-prey interactions. Dislodgement of aphids not only directly increased aphid mortality but also increased aphid consumption rates by ground-dwelling predators. Our results suggest that weather conditions such as wind and rain can modify predator-prey interactions in the field, presumably triggering synergistic effects. The combination of a manipulative microcosm experiment with a DNA-based gut content analysis proved to be an effective strategy which we recommend.

5 | DNA-based identification of scavenging and predation in a guild of generalist predators: a mesocosm study employing multiplex PCR

5.1. ABSTRACT

Ingestion of dead prey (scavenging) is supposed to be common in generalist predators, potentially causing errors in estimating predation rates by post-mortem techniques such as visual or molecular-based gut content analysis. We investigated consumption rates of a generalist predator guild of dead and living prey to evaluate the contribution of predation and scavenging in each predator species' diet. Replicated mesocosms (area 0.2 m^2), filled with potting compost covered with a layer of field soil were planted with wheat and infested with the grain aphid *Sitobion avenae*. Freshly killed individuals of the bird cherry-oat aphid *Rhopalosiphum padi* were given onto the soil surface and a generalist predator guild including carabids, staphylinids and spiders were released into each mesocosm. After two days predators were collected and prepared for DNA-based gut content analysis. We developed a multiplex approach to simultaneously amplify fragments of the mitochondrial cytochrome oxidase subunit I gene of the two aphid species in predator gut contents. All predators except the lycosid *Trochosa ruricolla* frequently consumed dead aphid prey, indicating commonness of scavenging in generalist predators. Interestingly, also the tetragnathid spider *Pachygnatha degeeri* consumed dead aphid prey at high rates. Consumption ratios of living and dead aphid prey differed between the predator species, suggesting two spider, one carabid and one styphylinid species as potential pest control agents. For the first time, a whole predator community could be tested for their feeding preferences concerning dead and living aphid

prey in model field arenas (mesocosms), encouraging further experiments to uncover potential errors in predation rate estimates for single predator species.

5.2. INTRODUCTION

Generalist predators feed on a wide range of prey, including herbivores (Symondson *et al.* 2002) and decomposers (Thiele 1977; Wise 1993; Krooss & Schaefer 1998). In agricultural systems feeding on herbivore populations may significantly reduce plant damage thereby increasing plant yield (Östman *et al.* 2003). To foster the control of herbivore populations by generalist predators the factors governing dynamics of predator and prey populations need to be understood (Symondson *et al.* 2002). Basicaly the trophic links in predator-prey systems need to be known. This is especially difficult in insect generalist predators, such as carabid and staphylinid beetles, as many of them are small, nocturnal or live subterranean. Post mortem gut content analysis is a promising tool to uncover the prey spectrum of generalist insect predators since the predators under investigation probably have been acting naturally prior to their capture (Sunderland 1988). Dissection and visual inspection of predators' gut content is possible (Ingerson-Mahar 2002) but only allows to trace prey leaving solid food remains in the gut of the predator, which often is not the case due to fluid feeding. Biochemical and molecular techniques overcome such restrictions and have been rapidly developed over the last two decades (reviewed in Symondson 2002; Sheppard & Harwood 2005; Sunderland *et al.* 2005). However, it has been stressed recently that these techniques cannot distinguish between prey that was scavenged or predated (Calder *et al.* 2005; Foltan *et al.* 2005; Juen & Traugott 2005). Therefore, molecular gut content analyses may overestimate the impact of predators on prey populations. As molecular techniques are becoming more frequently used to study predator-prey links in agricultural systems (Agusti *et al.* 2003; Harper *et al.* 2005; Greenstone *et al.* 2007; Juen & Traugott 2007), there is the need for investigating the relative importance of scavenging vs. predation in generalist predator species.

In wheat fields a high proportion of aphids has been found on the soil surface, and about 30 % of the aphids present were dead (Sopp *et al.* 1987). Therefore, dead and living prey is available for generalist predators. A wide range of generalist predator species consumes aphids (Sunderland & Vickerman 1980; Sunderland *et al.* 1987; Sunderland 2002). However, consumption of dead individuals has no pest control value (Sunderland 1996). Generalist predator guilds have been demonstrated to reduce aphid populations in the field (Edwards *et al.* 1979; Chiverton 1986; Helenius 1990; Dennis & Wratten 1991; Lang 2003; Schmidt *et al.* 2003), however, the predator species contributing to prey suppression are still little known.

Studies investigating prey choice (dead or alive) were mainly laboratory-based and were conducted in simple one-predator-prey Petri-dish systems (Tod 1973; Wolff 1986; Horne *et al.* 2000; Mundy *et al.* 2000; Lang & Gsödl 2001; Foltan *et al.* 2005). Results derived from such experiments may be questioned, as predators are confronted with their prey in a simplified environment and it is known that adding only simple structural elements alters the attack ratesof generalist predators (Rickers & Scheu 2005; O Vučić-Pestić *et al.*, unpublished data). Therefore, prey choice experiments in complex semi-natural systems are required; however, they are difficult to perform. Molecular techniques open up new possibilities also in mesocosm studies (Chapter 4), and we employed a PCR based predator gut content analysis in studying prey choice (live and dead) of a whole community of generalist predators in experimental arenas (mesocosms) consisting of a winter wheat system with two aphid species and a guild of generalist predators.

Two aphid species were introduced into the mesocosms: dead *Rhopalosiphum padi*, placed on the soil surface immediately before predator release, and living *Sitobion avenae*, introduced on wheat plants two weeks before the experiment started. Predator guts were screened for prey DNA using two species-specific primer pairs in a multiplex PCR targeting the mitochondrial cytochrome oxidase subunit I gene of *Rhopalosiphum padi* and *Sitobion avenae*, respectively. We hypothesised (i) that the generalist predator species feed on both dead and living aphid prey and (ii) that predator species differ in their preference for dead and living aphids.

5.3. MATERIALS AND METHODS

Predators

Generalist predators including carabids, staphylinids, and spiders were collected by pitfall trapping and hand searching from a winter wheat field near Darmstadt, Germany, during May and June 2006. Beetles were transferred individually into plastic containers (diameter 9.5 cm, height 4.5 cm) filled with damp potting compost and maintained in a controlled environment (16 °C, L:D 16:8) until the start of the experiment. Spiders were transferred individually into small plastic containers (diameter 6.5 cm, height 5.0 cm) with a damp piece of paper. All predators were fed with one dead *Calliphora vomitoria* larva, ten dead and five living *Drosophila melanogaster* adults twice a week to standardize the nutritional status of the predators. Living and dead prey were offered simultaneously to avoid conditioning of the predators to either one of these food types. Unconsumed carcasses were removed and replaced by freshly killed animals. Prior to the experiment, beetles and spiders were starved for five and nine days, respectively.

Experimental setup

The experiment was conducted in a ventilated greenhouse during June 2006. Mesocosms (diameter 50 cm, height 35 cm) were filled to three-quarters with potting compost covered with a 10 cm thick layer of field soil. The latter was taken from a ploughed crop field near Darmstadt in May 2006, sieved and heated to 60 °C for 3 h prior to the experiment to kill soil-living invertebrates. Coverage with field soil intended to simulate the soil surface structure of an arable field. The two soil layers were separated by a net (mesh size 1 mm) to prevent predators from entering the lower soil layer. Mesocosms were sown with winter wheat in four rows (ca. 15 cm distance between rows). After four weeks each mesocosm received about 70 aphids from a polyclonal population of *Sitobion avenae* F., cultured in glass containers on winter wheat at 24 °C and L:D 16:8. Mesocosms were immediately covered with mosquito nets (mesh size 1 mm, tightly sealed with tape) up to 75 cm in height. Aphids were allowed to settle and reproduce for two weeks. Then five mesocosms were randomly chosen receiving 21 predators each. Predator groups per mesocosm consisted of the carabids *Nebria brevicollis*

Fabricius (n = 2), *Notiophilus biguttatus* Fabricius (n = 1), *Platynus dorsalis* Pontopiddan (n = 4), *Poecilus cupreus* L. (n = 4) and *Pseudophonus rufipes* De Geer (n = 2); the staphylinids *Ocypus similis* Fabricius (n = 1) and *Philonthus fuscipennis* Mannh. (n = 4) and the spiders *Pachygnatha degeeri* Sundevall (n = 1) and *Trochosa ruricola* De Geer (n = 2). For predator introduction the nets were carefully lifted at one side of the mesocosms, the predators were set on the soil surface in the middle of the mesocosm and nets were immediately closed. Before predators were released, 100 dead individuals of *Rhopalosiphum padi*, cultured under the same conditions as *Sitobion avenae* and killed by freezing, were placed onto the soil surface of each of the five mesocosms. Aphids were spread in batches of ten individuals near the edge of the mesocosm in a starlike design with one batch in the middle of the mesocosm. Predators were allowed to feed for two days. Then nets were carefully removed, the wheat plants were cut and searched for predators. Subsequently, the soil surface and the upper soil layer were searched for predators. Predators were placed separately in Eppendorf tubes, frozen immediately and stored at -24 °C until DNA extraction. Five mesocosms selected at random were handled per day for nine consecutive days allowing to set up and harvest the 45 mesocosms of the experiment.

DNA extraction and PCR

Prior to DNA extraction the gut of each beetle was removed and homogenised in 50 µL PCR water except in the carabid beetle *Notiophilus biguttatus* where whole animals were used for DNA extraction, as was done for spiders. For each beetle's gut dissection separate gloves were used to avoid sample-to-sample contamination. As beetles often regurgitated as a defence reaction when they were transferred from mesocosms into the Eppendorf tubes, their gut was homogenized in the same tube the beetle was stored avoiding loss of any regurgitated material. Twenty-five µL of the homogenate or whole animals (*Notiophilus biguttatus* and the two spider species) were utilised for DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers instructions. The DNA was resuspended in 200 µL of manufacturer's elution buffer and stored at -24 °C.

For primer design, sequences of part of the cytochrome oxidase subunit I from several specimens of *Sitobion avenae* and *Rhopalosiphum padi* were analysed, and several primers

targeting *Sitobion avenae* and *Rhopalosiphum padi*, respectively, were designed using PrimerPremier (PREMIERE Biosoft Int., Palo Alto, CA, USA) following the guidelines for primer design given by Hawkins (1997). Primers were tested for their specificity using DNA of *Sitobion avenae* and *Rhopalosiphum padi* and of 14 invertebrate predator species including those used in the experiment. After testing all primers for performance in PCR, the primer pairs S155/A153 and S 157/A103 were selected for *Rhopalosiphum padi* and *Sitobion avenae*, respectively (Table 1).

Table 1 Primers (5´- 3´) designed from the cytochrome oxidase subunit I mtDNA of *Rhopalosiphum padi* (Rhop-pad) and *Sitobion avenae* (Sit-ave) and amplified product sizes.

Primer name	Primer sequence	Product size (bp)
Rhop-pad-S 155	GGA ACA GGA ACA GGA TGA ACA	111
Rhop-pad-A 153	TGA TGA GAT TCC TGC TAA ATG TAG A	
Sit-ave-S 157	TCA GTY GAT TTA ACT ATT TTT TCA T	257
Sit-ave-A 103	TCT CCT CCT CCT GCT GGA	

A multiplex PCR was optimised to analyse the predator extracts for the presence of *Sitobion avenae* and *Rhopalosiphum padi* DNA in one reaction. Amplifications were performed in 10 µL reactions containing 3 µL of extracted DNA, 0.5 × Multiplex PCR master mix (Qiagen) and 0.2 µM of primers S155 and A153 as well as 1 µM of primers S157 and A103. Amplifications were carried out in an Eppendorf Mastercycler Gradient PCR machine, cycling conditions were 15 min at 95 °C, 35 cycles of 30 s at 94 °C, 45 s at 63 °C, 90 s at 72 °C, and final elongation of 10 min at 72 °C. PCR water, as well as aphid and predator DNA, were included within each PCR to test for DNA carry-over contamination, false-negative and false-positive amplifications. PCR products were visualised on a 2 % agarose gels stained with ethidium bromide.

5.4. RESULTS

On average, 90 % of the carabid and staphylinid beetles added to the mesocosms were recaptured after the two day experimental period. In spiders numbers of recaptured individuals were considerably lower with 64 % in *Trochosa ruricolla* and 58 % in *Pachygnatha degeeri*.

Both primer pairs proved to be specific for the respective aphid species. In the multiplex PCR, fragments of DNA of both aphid species were amplifiable simultaneously in similar band strength in extracts containing DNA of both aphid species as well as in extracts additionally containing the predator DNA (Fig. 1). In some extracts a third band of about 320 bp was amplified, but due to the distinct difference in length to the 257 bp fragment of *Sitobion avenae*, these bands were clearly distinguishable from each other.

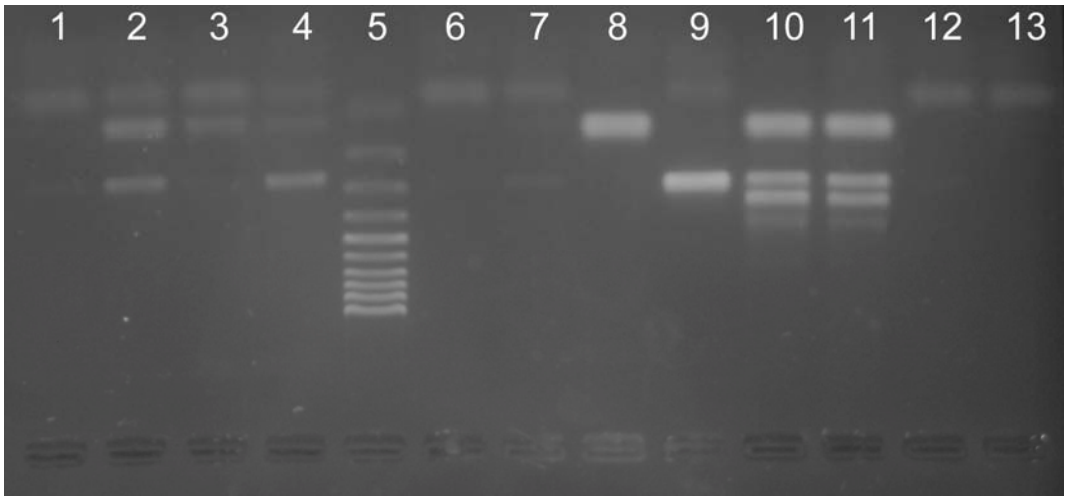

Fig. 1 Agarose gel electrophoresis of multiplex PCR amplification products using the primer pairs Rhop-pad-S 155/Rhop-pad-A 153 and Sit-ave-S 157/Sit-ave-A 103 targeting *Rhopalosiphum padi* and *Sitobion avenae*, respectively. This gel gives an example for screening the carabid *Nebria brevicollis* for aphid prey DNA. Lanes 1-4, 6-7 *N. brevicollis* retrieved from the experiment; lane 8 *R. padi*; lane 9 *S. avenae*; lane 10 *R. padi* + *S. avenae*; lane 11 *N. brevicollis* + *R. padi* + *S. avenae*; lane 12 *N. brevicollis* (starved); lane 13 distilled water; lane 5 100 bp DNA ladder (fermentas).

In the carabid species *Poecilus cupreus*, *Nebria brevicollis* and *Platynus dorsalis* significantly more beetles were tested positive for *R. padi* DNA than for *Sitobion avenae* DNA (Fig. 2). In contrast, in the two spider species *Trochosa ruricola* and *Pachygnatha degeeri*, significantly more individuals were tested positive for *Sitobion avenae* DNA than for *Rhopalosiphum padi* DNA. A similar pattern was also found for the carabid *Notiophilus biguttatus*, but the higher detection rates of *Sitobion avenae* DNA were only marginally significant. Two predators, the carabid *Pseudophonus rufipes* and the staphylinid *Philonthus fuscipennis*, showed similar detection rates for both *Rhopalosiphum padi* and *Sitobion avenae* DNA. In the staphylinid *Ocypus similis*, only one individual was tested positive for *Rhopalosiphum padi* DNA, therefore, no statistical analysis was possible for this predator. Rates of positive tested individuals for *Sitobion avenae* DNA strongly differed between the predator species, from 6 % in *Poecilus cupreus* to 67 % in *Pachygnatha degeeri*. In contrast, rates of positive tested predators for *Rhopalosiphum padi* only varied between 26 % in *Pseudophonus rufipes* and 41 % in *Platynus dorsalis*.

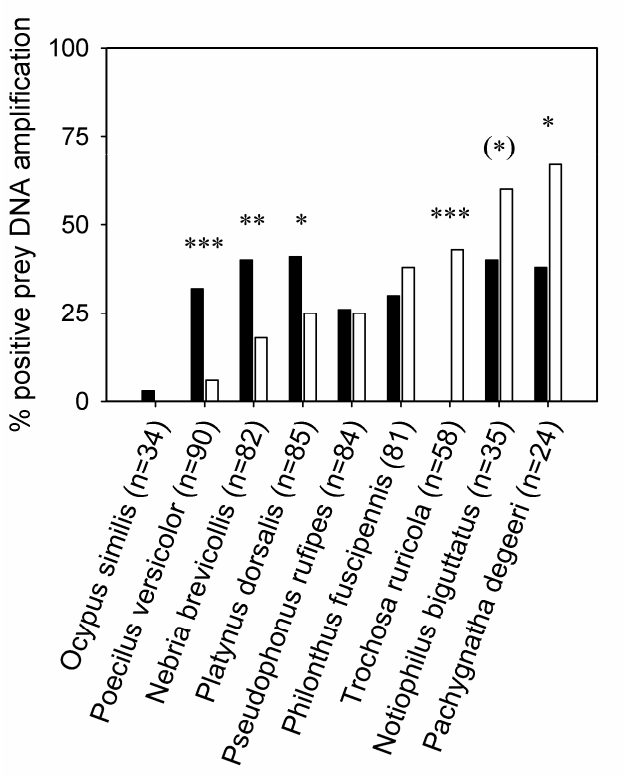

Fig. 2 Percentage of predators testing positive for DNA of dead (*Rhopalosiphum padi*, black bars) and living aphid prey *Sitobion avenae* (open bars). Significant differences are indicated (Chi-square tests; (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$). Numbers in brackets refer to the respective number of individuals tested from each predator species.

5.5. DISCUSSION

Both aphid species, *Sitobion avenae* and *Rhopalosiphum padi*, could be detected by multiplex PCR in predator extracts after the two day experimental period, confirming that predators interacted with their prey in the 0.2 m^2 mesocosms. Despite aphids are of low food quality for generalist predators (Bilde & Toft 1994; Toft 1995; Toft 1997), consumption rates in the field can be high (Sunderland & Vickerman 1980; Sunderland *et al.* 1987; Harwood *et al.* 2005); also in our mesocosms predators heavily fed on aphids. As our mesocosms were not exposed to wind or rain, the fraction of *Sitobion avenae* being active on the ground presumably was negligible. Therefore, the dead aphid prey, *Rhopalosiphum padi*, could be solely consumed at the soil surface, whereas the living aphid prey, *Sitobion avenae*, presumably was consumed mainly in the vegetation.

The predator screening by PCR showed positive tested individuals for the dead aphid prey (*Rhopalosiphum padi*) in all predator species except the lycosid *Trochosa ruricola*. With the exception of *Ocypus similis*, between 26 % and 41 % of the tested individuals in each predator species had consumed dead aphid prey. This indicates that dead prey forms a considerable part of nutrition for a wide range of generalist predators. Dead prey represents an easily available (Lang & Gsödl 2001) source of energy and nutrition, and most carabids exploit dead prey when available (Symondson 2002). In addition to carabids, also the staphylinid *Philonthus fuscipennis*, an abundant species in agricultural fields (Dennis & Wratten 1991; Krooss & Schaefer 1998), consumed dead aphids, with rates of positive tested specimens being in the range of those of the carabid beetles.

Interestingly, also one of the two spider species investigated, the tetragnathid *Pachygnatha degeeri*, had consumed dead aphids. Generally, spiders are thought to rarely scavenge, but some studies showed consumption of dead prey in non web-building spiders (Knost & Rovner 1975; Aitchison 1984; Wolff 1986). Nentwig (1987) stated that scavenging may significantly contribute to the diet of non web-building spiders and in fact dead aphids were consumed by 38 % of the specimens of *Pachygnatha degeeri* in our experiment. In contrast, none of the individuals of the lycosid *Trochosa ruricola* were tested positive for dead aphid DNA,

indicating differences in the acceptance of dead aphid prey between non web-building spider species.

In contrast to consumption of dead aphid prey, predators strongly differed in detection rates for the living aphid prey DNA. Predators could be classified in three groups. In the first group, consisting of *Poecilus cupreus*, *Nebria brevicollis* and *Platynus dorsalis*, significantly more individuals in each predator species were tested positive for *Rhopalosiphum padi* than for *Sitobion avenae*, indicating preferences for dead aphid prey. Griffiths *et al.* (1985) showed for *Platynus dorsalis* a mainly ground searching behaviour without the ability to climb wheat plants. This corresponds to our results of higher detection rates of the dead soil surface exposed aphid prey in *Platynus dorsalis*. Therefore, a mainly ground searching behaviour can also be assumed in the other two carabid beetles (*but see* Mundy *et al.* 2000). In the second group, consisting of the carabid *Pseudophonus rufipes* and the staphylinid *Philonthus fuscipennis*, similar rates of predator specimens were tested positive for both *Sitobion avenae* and *Rhopalosiphum padi*, suggesting that these beetles consume aphids irrespective if they are dead or alive. *Pseudophonus rufipes* has been shown to prey on *Sitobion avenae* and *Rhopalosiphum padi* (Sunderland *et al.* 1987; Kielty *et al.* 1999), and *Philonthus fuscipennis* is known to be a voracious predator of *Sitobion avenae* (Dennis & Wratten 1991). Nevertheless, as indicated by our results, both predators also consume dead aphid prey in similar rates to living ones. In the third predator group, significantly more individuals in each predator species (*Trochosa ruricola*, *Notiophilus biguttatus* and *Pachygnatha degeeri*) were tested positive for the living aphid prey, indicating that these species mainly act as predators by searching for prey in the vegetation. The lycosid *Trochosa ruricola* exclusively fed on living aphid prey; concordantly lycosids were found to contribute significantly to aphid suppression in a winter wheat field (K Birkhofer *et al.*, unpublished data). *Pachygnatha degeeri* has been previously shown previously to consume aphids at high rates (Harwood *et al.* 2005), and also in our experiment 67 % of the individuals of this spider consumed *Sitobion avenae*. The carabid species *Notiophilus biguttatus* has been proven before to consume aphids using molecular techniques (Sunderland *et al.* 1987), but its role in aphid suppression has not been evaluated yet. Our results suggest that this species is a potential aphid control agent, consuming aphids at high rates.

A ranking list of the generalist predators in relation to their aphid consumption rates is difficult to gain, as it has been shown that detectability of prey DNA strongly differs between different predator species (Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Ma *et al.* 2005; Read *et al.* 2006; Greenstone *et al.* 2007). The high aphid DNA detection rates in the two spider species likely resulted at least in part from prolonged prey DNA detectability in spiders (Sheppard *et al.* 2005; M Traugott & WOC Symondson, unpublished data), which likely is due to their ability to lower metabolic rates in response to starvation (Anderson 1970) and/or the usage of gut diverticula to store partially digested food (Nakamura & Nakamura 1977). Results from enzyme linked immunosorbent assay (ELISA) suggest that staphylinids digest prey proteins faster than carabids and spiders (Sunderland *et al.* 1987), and this likely also applies to DNA. Therefore, the high detection rates of aphid prey DNA in *Philonthus fuscipennis* in our study possibly still underestimates the consumption of aphids by this predator. Also, comparisons of detection rates among carabid beetle species are challenging. Using antibodies to measure digestion of prey proteins in two related species of carabid beetles Symondson and Liddell (1993b) found large differences in digestion rates between the smaller *Pterostichus madidus* and the larger *Abax parallelepipedus*. Moreover, we found significant differences in detection rates of aphid DNA between two closely related carabid beetles, *Pterostichus melanarius* and *Nebria brevicollis* (Chapter 6).

The fact that DNA gut content analysis can not distinguish between scavenging and predation proved to be very useful in our experiment. For the first time, a whole predator community could be tested for their feeding preferences concerning dead and living aphid prey in model field arenas (mesocosms). Different predator groups could be identified to be rather scavengers or predators. With the exception of the lycosid *Trochosa ruricola*, none of the predator species exclusively fed on living aphids but rather included dead prey in their diet. These findings support previous assumptions that generalist predators are facultative scavengers (Sunderland 1996; Symondson *et al.* 2002), and this needs to be considered for interpreting data from molecular gut content analyses of field caught generalist predators.

6 | The effects of temperature on detection of prey DNA in two species of carabid beetles

6.1. ABSTRACT

PCR-based techniques to investigate predator-prey trophic interactions are starting to be used more widely but still factors affecting DNA decay in predator guts are poorly understood. Here we investigated the effects of time since feeding, temperature and amplicon size on the detectability of prey DNA in the gut content of two closely related predator species. Cereal aphids, *Sitobion avenae*, were fed to the carabid beetles *Pterostichus melanarius* and *Nebria brevicollis*. Beetles were allowed to digest their meal at 12 °C, 16 °C and 20 °C and batches of beetles were subsequently frozen at time periods from 0-72 h after feeding. Aphid DNA was detected within beetles' gut contents using primers amplifying fragments of 383 bp, 317 bp, 231 bp and 85 bp. Prey DNA detection rates were significantly higher in *Nebria brevicollis* than in *Pterostichus melanarius*, indicating fundamental dissimilarities in prey digestion capacities. High temperatures (20 °C) and large amplicons (383 bp) significantly decreased detection rates. The shortest amplicon gave the highest prey DNA detection success, whereas no differences were observed between the 317 bp and the 231 bp fragment. Our results indicate that factors such as ambient temperature, predator taxon and amplicon size should all be considered when interpreting data derived from PCR-based prey detection. Correction for such factors should make calculation of predation rates in the field more accurate and could help us to estimate when predation events occur in the field.

6.2. INTRODUCTION

Predator-prey interactions are important processes driving animal population dynamics and are central to many ecological studies. Identification of trophic links can be difficult without disturbing the system under study, especially in predators which are small, active at night or living in the soil. *Post mortem* determination of predator diets, using gut content analysis, is an accurate method as the predators can be assumed to have been behaving naturally prior to their capture. Visual examination of predator gut contents is possible, but requires the intake of recognisable prey compartments by the predator (Ingerson-Mahar 2002). As most invertebrate predators are at least partly fluid feeders many trophic links are inevitably missed using this approach. Biochemical and molecular techniques overcome these problems and have been rapidly developed over the last two decades (reviewed in Symondson 2002; Sheppard & Harwood 2005; Sunderland et al. 2005). PCR-based techniques of *post-mortem* gut content analysis have been widely used and applied to insect predator-prey systems including Coleoptera, Diptera, Heteroptera, Homoptera, Lepidoptera, Neuroptera and Collembola but also Annelida, Crustacea, Arachnida and Mollusca (Harper *et al.* 2005; Read *et al.* 2006; Gariepy *et al.* 2007; Juen & Traugott 2007). Although several studies have investigate parameters that might affect detection periods and amplification success (Agusti *et al.* 1999; Zaidi *et al.* 1999; Chen *et al.* 2000; Hoogendoorn & Heimpel 2001), several factors remain to be explored, such as the effect of ambient temperature and predator taxon. The more we know about which factors affect prey DNA detection success, the better we will be able to interpret field-derived data and assess trophic links and their strength in natural systems.

One of the fundamental parameters affecting prey DNA detectability is the time elapsed since feeding (Symondson 2002). In general, DNA detectability decreases with increasing digestion time, but considerable differences between predator species have been reported (Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Ma *et al.* 2005; Read *et al.* 2006; Greenstone *et al.* 2007; M Traugott & WOC Symondson submitted). Furthermore, Hoogendorn and Heimpel (2001) showed that an increase of ambient temperature significantly decreases prey DNA detectability within coccinellid predators, indicating that

temperature influences the rate of DNA digestion. Likewise, the size of the target DNA molecule influences the amplification success of prey DNA. This was first recognised by Zaidi *et al.* (1999) who showed longer post-feeding detection of a smaller amplicon for up to 28 h, compared with larger amplicons that were rapidly degraded.

In the present study we investigated whether ambient temperature and fragment length affect the post-feeding detectability of prey DNA in two different but closely related predator species, in a full-factorial experimental design. We hypothesised that (i) the detectability of prey DNA differs even between closely related predator species and (ii) that the post-feeding prey detection period is affected by ambient temperature and target fragment size. Based on these results, we discuss whether PCR-based prey detection might allow us to calculate the time at which predation occurred in the field.

6.3. MATERIALS AND METHODS

Insects

During October 2005, adult carabid beetles, *Nebria brevicollis* (F.) and *Pterostichus melanarius* (Ill.), were collected by pitfall trapping and hand searching from two fields at Burdens farm, Wenvoe, near Cardiff, UK. Beetles were transferred individually into plastic containers (8.5 cm diameter, 4.5 cm height) filled with 80 g moist sphagnum peat and maintained in a controlled environment (L:D 16:8; 16 °C). They were fed with one *Calliphora vomitoria* (L.) larva twice a week. Prior to the feeding experiments, beetles were starved for five days to ensure the same nutritional status in all individuals. Grain aphids, *Sitobion avenae* (F.), were reared on wheat plants in fine mesh cages. From these cultures adult aphids were removed and frozen as prey for the subsequent feeding experiments at -80 °C.

Feeding experiments

Feeding experiments were carried out in controlled climate chambers at 12 °C, 16 °C, and 20 °C (L:D 16:8). Experiments were conducted separately for the two carabid species but simultaneously at all three temperature levels for each species. Petri dishes lined with filter paper were used as feeding arenas. Within each arena one carabid beetle was allowed to feed for 1 h on five freeze-killed adult aphids. Beetles consuming less than three aphids were discarded from the experiment. After the on-hour-feeding period aphid remains were removed and arenas provided with fresh filter paper. Additionally, a piece of damp filter paper was added, serving as a shelter for the beetles. Beetles were frozen at -80 °C after digesting their meal for 0, 3, 6, 12, 24, 36, 48 and 72 h from the end of the feeding period on. At each time point post-feeding seven individuals were frozen, except for *Pterostichus melanarius* at 12 °C/0 h (n=6) and *Nebria brevicollis* at 12 °C/72 h (n=6), at 16 °C/72 h (n=6) and at 20 °C/72 h (n=5). Due to problems with maintaining *Nebria brevicollis* at 20 °C for extended time periods post feeding (36 h, 48 h, and 72 h), an additional set of beetles was used for these three time points where carabids were kept in Petri dishes filled with damp soil.

Sequencing and primer design

For sequencing, DNA of aphids was extracted using a Chelex protocol. Whole aphids, *Sitobion avenae*, where homogenised separately in 20 µL PBS, mixed with 5 µL Proteinase K and 200 µL 10% Chelex solution and incubated at 56 °C for 4 h on a rocking platform. After a final incubation at 94 °C for 15 min samples were stored at -24 °C. Universal invertebrate primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994) were used to amplify part of the mitochondrial cytochrome oxidase subunit I gene. PCR was carried out in a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) in 20 µL reaction volumes containing 3 µL of extracted DNA, 0.25 mM dNTPs (Invitrogen GmbH, Karlsruhe, Germany), 1 µM of each primer, 2 µL 10 × buffer (Invitrogen), 3 mM $MgCl_2$, and 1.5 U *Taq* DNA polymerase (Invitrogen). Initial denaturation was done at 94 °C for 2 min, followed by 35 cycles at 94 °C for 15 s, 50 °C for 30 s, 72 °C for 45 s, and final elongation at 72 °C for 2 min. PCR products were purified using ExoSAP-IT (USB, Staufen, Germany), subjected to sequencing PCR using Big-Dye Terminator mix (version 1.3, Applied Biosystems) and

sequenced in both forward and reverse directions. Sequences were aligned using BioEdit (Hall 1999) and corrected manually.

Five forward (S101 – S105) and three reverse primers (A101 – A103) were designed using PrimerPremier (PREMIERE Biosoft) following the guidelines for primer design given by Hawkins (1997). The resulting 15 primer pair combinations were tested for their sensitivity using DNA from *S. avenae* and for their specificity using DNA from the two carabid species. Optimisation of the PCR protocol included determination of optimum annealing temperatures by temperature gradient PCR, testing different concentrations of primers and adjusting cycling conditions.

Screening predators for prey DNA

The beetles from the feeding trials were thawed, their foreguts removed and homogenised in 50 µL of PCR water using a plastic pistil. For each beetle separate gloves were used to avoid sample-to-sample contamination. As beetles often regurgitated as a defence reaction when they were transferred from the feeding arenas into the Eppendorf tubes, their gut was homogenized in the same tube the beetle was stored in, avoiding loss of any regurgitated material. Twenty-five µL of the homogenate were used for DNA extraction with DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions and 200 µL of the DNA extracts were stored at -24 °C.

The extracts were analysed for the presence of aphid DNA using four primer pairs (Table 1) amplifying fragments from 85 to 383 bp. PCRs were performed in 10 µL reactions containing 3 µL of extracted DNA, 0.25 mM dNTPs (Fermentas GmbH, St. Leon-Rot, Germany), 1 µM of each primer, 1 µL 10 × buffer (Invitrogen), 3 mM $MgCl_2$, 0.12 µg bovine serum albumin (BSA) and 1.5 U *Taq* DNA polymerase (Invitrogen). Amplifications were carried out in an Eppendorf Mastercycler Gradient PCR machine, cycling conditions were 2 min at 94 °C, 40 cycles of 15 sec at 94 °C, 30 sec at 61 °C, 45 sec at 72 °C, and final elongation of 2 min at 72 °C. PCR water, as well as aphid and carabid DNA, were included within each PCR to test for DNA carry-over contamination, false-negative and false-positive amplifications. PCR products were visualised on a multi-channel capillary gel electrophoresis system HDA-GT12 (eGene, Inc., Irvine, CA, USA).

Statistical analysis

Chi-square tests were performed to test for differences in detection rates for aphid DNA within each of the three temperature levels and each of the four fragment lengths between the two carabid species. To investigate the effects of temperature, fragment length and digestion time on detectability of aphid DNA within both predator species, a three-variable logistic regression was performed including prey DNA detectability (yes/no) as the dependent variable and temperature (12 °C; 16 °C; 20 °C), fragment length (383 bp; 317 bp; 231 bp; 85 bp), and time since feeding (0 h; 3 h; 6 h; 12 h; 24 h; 36 h; 48 h; 72 h) as the independent variables. A logistic regression model was chosen because of the dichotomous and nominal character of the response variable. As the design is experimental and the independent variables are not correlated, we were testing all variables simultaneously instead of using variable selection methods (Cody & Smith 2006). In case of significant effects of independent variables, single logistic regressions were calculated comparing two factor levels at a time in all possible combinations within the variable, equivalent to performing protected ANOVAs following a significant MANOVA (Scheiner & Gurevitch 2001). Logistic regressions and Chi-square tests were calculated using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and STATISTICA 7.1 (StatSoft, Tulsa, USA), respectively. For the *Nebria brevicollis* feeding experiment at 20 °C, all statistics were calculated without data from digestion times 36 h, 48 h, and 72 h. Instead of calculating the detectability half-life as described by Greenstone and Hunt (1993), using an exponential model, the real time points as defined by the experimental design were determined at which more than 50% of the beetles were tested positive for aphid DNA.

6.4. RESULTS

The four primer pairs S101/A103, S102/A103, S103/A103 and S105/A103 successfully amplified DNA fragments of *Sitobion avenae* of 383, 317, 231 and 85 bp, respectively. The optimal annealing temperature identified by temperature gradient PCR was 61°C for all four primer pairs. All amplifications were optimised to run at the same cycle conditions and PCR reagent concentrations. The PCR assay proved to be (for our purposes) specific for *Sitobion avenae* DNA as no amplicons were obtained with DNA of the two carabid species.

Table 1 Primers (5´- 3´) designed from the cytochrome oxidase subunit I mtDNA of *Sitobion avenae* and expected product sizes of each forward primer combined with the reverse primer Sit-ave-A103.

Primer name	Primer sequence	Product size (bp)
forward		
Sit-ave-S 101	att aga ttt tga yta cta cca cca	383
Sit-ave-S 102	aca ggt aca gga tga act att tac	317
Sit-ave-S 103	aca ttt agc agg aat ctc atc a	231
Sit-ave-S 105	tac cag ttt tag ctg gtg ct	85
reverse		
Sit-ave-A103	tct cct cct cct gct gga	

Detectability rates of aphid DNA in the predators' gut contents differed significantly between *Pterostichus melanarius* and *Nebria brevicollis*, with overall detection rates being significantly higher (χ^2 = 41.63; P < 0.001) in *Nebria brevicollis* (61%; n = 580) than in *Pterostichus melanarius* (42%; n = 584). These higher detection rates in *Nebria brevicollis* were significant within each of the three temperature levels and each of the four fragment lengths (Table 2).

Table 2 Results of cross-tabulation tables testing for differences in DNA detectability of aphid prey between *Pterostichus melanarius* and *Nebria brevicollis* with respect to fragment length and temperature

	χ^2 df = 1	*P*
383 bp	12.89	< 0.001
317 bp	13.64	< 0.001
231 bp	10.39	0.001
85 bp	9.46	< 0.01
12 °C	8.18	< 0.01
16 °C	7.06	< 0.01
20 °C	38.83	< 0.001

Detectability of aphid DNA in *Pterostichus melanarius* significantly decreased with increasing digestion time, temperature and fragment length (Table 3). Mean detection rates were similar at 12 °C (44 %) and at 16 °C (43 %) but were significantly lower at 20 °C (29 %) (Table 4). These lower detection rates were due to a rapid decline in detectability of aphid DNA within digestion times 0 – 12 h at 20 °C compared to a moderate decline in detectability in beetles maintained at 12 °C and 16 °C (Fig. 1a).

Table 3 Summary of logistic regression analysis for the effect of temperature (temp), fragment length (frag length) and time on aphid DNA detectability in the guts of *Pterostichus melanarius* (n = 668) and *Nebria brevicollis* (n = 420)

	Pterostichus melanarius			*Nebria brevicollis*		
factor	Wald χ^2	df	*P*	Wald χ^2	df	*P*
time	81.02	7	< 0.001	19.04	7	< 0.001
temp	16.05	2	< 0.001	0.005	2	*n.s.*
frag length	37.58	3	< 0.001	13.52	3	< 0.01
temp*frag length	1.78	6	*n.s.*	7.62	6	*n.s.*
temp*time	25.81	14	0.027	18.08	14	0.021
frag length*time	15.68	21	*n.s.*	8.51	21	*n.s.*

Table 4 Results of single logistic regressions for the effect of each factor level combination of the factors temperature and fragment length on aphid DNA detectability in the guts of *Pterostichus melanarius* and *Nebria brevicollis*

	Pterostichus melanarius		*Nebria brevicollis*	
	Waldχ^2(1)	*P*	Waldχ^2(1)	*P*
12°C vs. 16°C	0.03	*n.s.*	…	…
12°C vs. 20°C	11.43	< 0.001	…	…
16°C vs. 20°C	10.44	0.001	…	…
383 bp vs. 317 bp	9.92	0.0016	8.10	< 0.001
383 bp vs. 231 bp	10.62	0.001	5.80	0.016
383 bp vs. 85 bp	60.03	< 0.001	25.25	< 0.001
317 bp vs. 231 bp	0.01	*n.s.*	0.21	*n.s.*
317 bp vs. 85 bp	25.73	< 0.001	6.55	0.01
231 bp vs. 85 bp	24.68	< 0.001	8.84	0.003

In *Nebria brevicollis* prey DNA detection rates significantly decreased with increasing fragment length and digestion time. Detection rates differed significantly between the three temperature levels depending on digestion time. Detectability of aphid DNA decreased markedly between 6 h and 12 h and between 12 h and 24 h at 20 °C and 16 °C, respectively, compared to a more constant detectability of aphid DNA up to 24 h in beetles maintained at 12 °C (Fig. 1b).

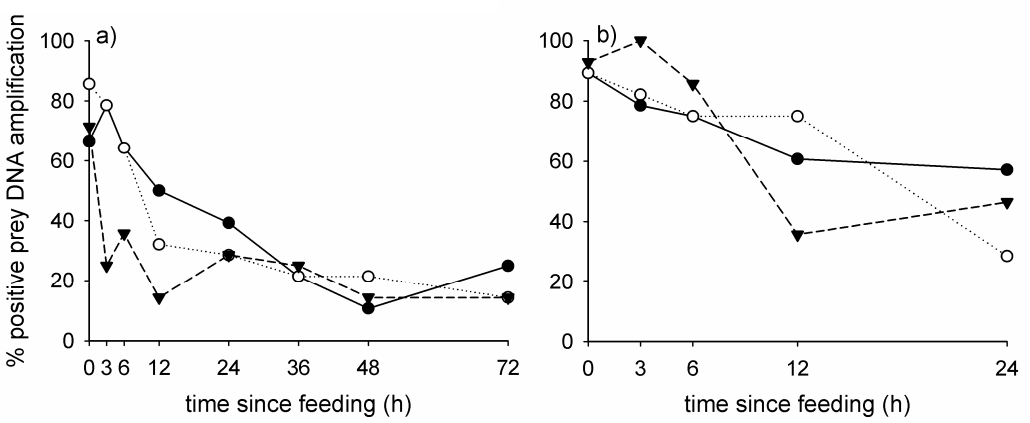

Fig. 1 Mean aphid prey DNA detection rates (%) in the gut of a) *Pterostichus melanarius* and b) *Nebria brevicollis* up to 72 h post-feeding of beetles kept at 12°C (–●–), 16°C (-○-) and 20°C (–▼–)

A clear effect of fragment length on prey detection in *Pterostichus melanarius* was observed: for the three larger fragments (383 bp, 317 bp, 231 bp) amplification success decreased below 50 % between 0 h and 24 h post-feeding at all temperature levels (Fig. 2a). In contrast, the shortest fragment (85 bp) was detectable in over 50 % of the beetles up to 24 h, 36 h and 72 h at 20 °C, 16 °C and 12 °C, respectively. Prey DNA detection rates differed significantly among the different-sized amplicons except for the 317 bp and 231 bp fragments (Table 4).

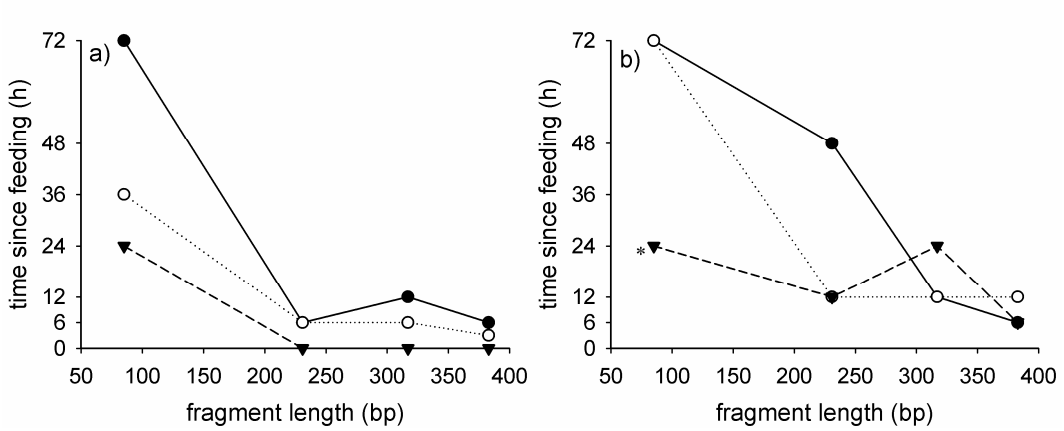

Fig. 2 Maximum time since feeding with more than 50% positive tested beetles for the four different sized amplicons (85, 231, 317 and 383 bp) of aphid DNA at 12°C (–●–), 16°C (-○-) and 20°C (–▼–) in a) *Pterostichus melanarius* and b) *Nebria brevicollis*. *Data for the detection times of aphid DNA in *Nebria brevicollis* at 20°C only available up to 24 h

In *Nebria brevicollis* the effect of fragment length on prey detection was more distinct between temperatures than in *Pterostichus melanarius*. The two larger fragments (383 bp, 317 bp) were successfully detectable in more than 50 % of the beetles up to between 6 h and 24 h digestion time at all temperatures (Fig. 2b). For the second shortest fragment (231 bp), 50% amplification success was similar at 16 °C and 20 °C (12 h), whereas even at 48 h post feeding more than 50 % of the beetles tested positive at 12 °C. For the shortest fragment (85 bp) more than 50 % of the beetles still tested positive after 72 h at 12 °C and 16 °C and up to 24h at 20 °C. Similar to the results in *Pterostichus melanarius*, detection rates among the different-sized amplicons in *Nebria brevicollis* differed significantly except for the 317 bp and 231 bp fragments (Table 4).

6.5. DISCUSSION

Within the present study we found that aphid prey DNA detection rates differed significantly in two carabid species, with a higher detectability in *Nebria brevicollis* compared to *Pterostichus melanarius*. It has been shown in previous studies that detection rates of prey protein (Sunderland *et al.* 1987; Symondson & Liddell 1993a; Hagler & Naranjo 1997) and prey DNA (Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Ma *et al.* 2005; Read *et al.* 2006; Greenstone *et al.* 2007) can differ considerably in different predator species. Results from enzyme linked immunosorbent assay (ELISA) suggest that staphylinids digest prey proteins faster than carabids and spiders (Sunderland *et al.* 1987). The spiders' prolonged detection times for prey protein and prey DNA (Harwood *et al.* 2004; Sheppard *et al.* 2005; M Traugott & WOC Symondson, unpublished data) are possibly due to their ability to vary their metabolic rates in response to starvation (Anderson 1970) and/or to the usage of their gut diverticula to store partially digested food (Nakamura & Nakamura 1977). The longer detection times in carabids, compared with staphylinids, may possibly result from the intake of solid prey remains together with fluids, whereas rove beetles are mainly fluid feeders (Sunderland & Vickerman 1980; Lövei & Sunderland 1996). Note that fluid-feeding carabid larvae were found to have extended prey DNA detection times as well, which, however, were significantly influenced by prey species (Juen & Traugott 2005; 2006; 2007). Comparing detectability of potato beetle DNA in a pentatomid bug and ladybeetle larvae, Greenstone *et al.* (2007) found significantly higher mean prey DNA detection times in the former which has been ascribed to the bug's spider-like hunting style and feeding mode. In contrast to these studies we here compared, for the first time using PCR, detection rates of the same prey species in taxonomically closely related predators. A similar study using antibodies to measure digestion of prey proteins in two related species of carabid found large differences in digestion rates between the smaller *Pterostichus madidus* and the larger *Abax parallelepipedus* (Symondson & Liddell 1993b). The carabid beetles *Pterostichus melanarius* and *Nebria brevicollis* are both night-active autumn breeders which hunt on the soil surface and have a similar feeding mode (Williams 1959; Greenslade 1963; Chapman *et al.* 1999). Despite these analogies, considerable differences in prey DNA detection rates were found

between these two predator species. Interestingly, these differences were not altered by ambient temperature or prey amplicon length, indicating a fundamental dissimilarity in prey digestion capacities. These findings underscore the need to assess prey DNA detectability not only for predator taxa showing different feeding modes (Chen *et al.* 2000; Greenstone *et al.* 2007), but also for closely related species sharing the same feeding mode to allow correct interpretation of field-derived data.

The present experiment showed that DNA detection success in *Nebria brevicollis* prey was negatively correlated with increasing ambient temperature, whereas in *Pterostichus melanarius* prey detection rates were significantly reduced only at 20 °C, compared to rates at 12 ° and 16 °C. This suggests a non linear relationship between DNA digestion rates and ambient temperature for this predator-prey system. In accord with this finding, D Read et al. (unpublished data) found that only temperatures above 20 °C affected detection rates of nematode prey DNA in the guts of the collembolan *Folsomia candida*, whereas no significant differences in mean detection times occurred between ambient temperatures ranging from 4 °C to 16 °C. Significantly higher DNA detectability rates of lepidopteran eggs in the ladybird *Coleomegilla maculata* were observed at 20 °C compared to 27 °C ambient temperature (Hoogendoorn & Heimpel 2001). These results indicate that the effects of temperature on prey digestion depend on the specific predator-prey system investigated and the environmental conditions within which these trophic interactions happen. Perhaps temperature effects can be neglected in systems where ambient temperatures fluctuate only within a small range, e.g. in soil-dwelling predators (Juen & Traugott 2007) or epigeic predators hunting under a dense plant canopy. In contrast, effects of ambient temperature on prey DNA digestion rates need to be considered in predators which are exposed to considerable temperature fluctuations. Clearly, further experimental work is needed on this topic to allow better interpretation of field-derived data on prey DNA detection rates.

Within the present study we found that in most cases prey detection rates were positively correlated with decreasing prey DNA fragment length. Several studies have shown that this relationship holds true also in other predator-prey systems, with short amplicons allowing detection of prey DNA for longer periods post feeding (Agusti *et al.* 1999; Zaidi *et al.* 1999; Hoogendoorn & Heimpel 2001). However, we also found within the present experiments that

prey DNA detection rates were not significantly different between the 317 bp and the 231 bp fragment. Similarly, Chen *et al.* (2000) found no differences in detectability half-lives for fragments of 246 bp and shorter; and no differences in detection rates of DNA fragments between 127 and 585 bp were found within 24 h post feeding intervals in carabid larvae-scarabaeid larvae predator-prey systems (Juen & Traugott 2005; 2006; 2007). These results indicate that the efficiency of PCR to amplify semi-digested prey DNA fragments is, besides amplicon length, also determined by factors such as the quality of the template DNA extract, PCR reagents, cycle conditions and the efficiency of the primers. In our study, the impact of those effects was diminished by optimising all primers to amplify at the same cycle conditions, using the same PCR reagent concentrations and by combining all forward primers with the same reverse primer. Therefore, differences in detection rates can mainly be ascribed to the factors investigated, strengthening the significance of our results.

By using a set of fragments that can be detected for different lengths of time, Hoogendoorn and Heimpel (2001) aimed to estimate the time since feeding in a ladybird beetle-lepidopteran egg predator-prey system. The authors suggested that sequences increasing in length can be used to estimate a minimum and a maximum time since the predators consumed prey. This approach, however, demands a clear relationship between fragment length and amplification success at post feeding intervals. Considering the results of the present study and of those discussed above, a set of primers amplifying fragments of distinct differences in amplicon size should be used to estimate the time point since feeding. The resolution of the time scale derived by the amplification of such fragments, however, is suspected to be somewhat restricted. Nevertheless, ecologically relevant questions could be answered, for example differentiation between night and diurnal predator activity or temporal niche differentiation of predators.

7 General Discussion

Predator-prey interactions are important processes driving animal population dynamics and are central to many ecological studies. Beside biotic factors such as predation and competition animal numbers are driven by abiotic factors (Krebs 2001; Begon *et al.* 2005), and these factors do not work independently; rather, they interact to affect population dynamics. In agricultural systems those effects are of major interest as strong predatory effects on herbivore prey may significantly reduce plant damage thereby increasing plant yield (Östman *et al.* 2003). Therefore, generalist predators can provide economically important ecosystem services.

In the present work I investigated predator-prey interactions in the agricultural model system winter wheat. Factors presumably affecting multitrophic interactions were addressed in two manipulative field experiments, one mesocosm experiment and one microcosm experiment. Furthermore, as molecular techniques were employed for the latter two experiments, laboratory feeding trials were conducted to advance the applicability of these techniques for field studies.

7.1. PREDATOR-PREY INTERACTIONS

A series of field studies has been carried out to investigate biological control effects of generalist predators with sometimes contradicting results; however, there is increasing evidence that generalist predators can reduce pest numbers in agricultural fields (reviewed in Symondson *et al.* 2002). In both of the field studies presented here we could demonstrate significant aphid suppression by a community of generalist predators (**Chapter 2**, **Chapter 3**), in some fields below the economic threshold (**Chapter 3**). However, also in our field

experiments, significant effects on prey populations were partially absent (**Chapter 2**), calling for the identification of factors possibly affecting predator-prey interactions.

Alternative prey: One important feature in generalist predators is their catholic feeding habit which enables them to feed on alternative prey in times when pest prey is scarce (Symondson *et al.* 2002). At pest arrival this enables generalist predators to be already in the fields which contrasts specialist predators, whose life cycle is adapted to their pest prey/host. However, biocontrol can fail if predators do not switch to pest prey due to a more palatable or manageable alternative prey. We could demonstrate successful aphid control in presence of alternative prey in the field (**Chapter 3**). Generalist predators fed on collembolan and aphid prey simultaneously, thereby reducing aphid numbers below the threshold level of economic damage. Moreover, with increasing aphid densities, generalist predators shifted from belowground prey to the aphid prey. This result is surprising as aphids have been shown to be of low food value or even toxic for many generalist predators (Toft 2005), theoretically tempering aphid control. Fostering alternative prey from the detritivore system therefore should increase biological control, as we proved both feeding on decomposers and shifting to aphid prey by generalist predators (**Chapter 3**). Triggering biological control through detrital subsidies has been investigated before in rice (Settle *et al.* 1996) and vegetable garden systems (Halaj & Wise 2002). In both studies positive effects on generalist predators could be demonstrated, but effects on pest populations were obscure. By mulching one hectare sized areas in wheat fields we could demonstrate strong utilisation of maize mulch by the decomposer subsystem (**Chapter 2**). Densities of some predator species were increased in the mulched plots and stable isotope analyses indicated foraging of these predators in both the detritivore and the herbivore system, thereby linking these two subsystems (‘dual subsystem omnivory’, Scheu 2001). However, it remains puzzling why the epigeic collembolan community was unaffected by mulching as well as the fact that successful aphid control was only present in two of the three fields. Nevertheless, the present field experiments demonstrated some features being essential for managing arable fields to foster biological control: (i) strong utilisation of a detrital subsidy by the decomposer community reaching into the below- and aboveground predator system (**Chapter 2**), (ii) increased predator densities in

mulched fields (**Chapter 2**), (iii) complementary feeding of decomposers and herbivores by generalist predators, thereby efficiently reducing herbivore populations (**Chapter 3**), (iv) generalist predator shifting from alternative prey to herbivore prey at increasing herbivore densities (**Chapter 3**), and (v) increased aphid control in mulched fields (**Chapter 2**).

Target prey density: It has been stressed that pest suppression by generalist predators should be strongest early in the year when pest populations are low (Edwards *et al.* 1979; Chiverton 1986). However, we could demonstrate aphid suppression even at high aphid densities in the field (**Chapter 3**), suggesting that other factors than aphid density may trigger generalist predator effects early in the year.

Abiotic factors: It has been shown that adverse weather can significantly decrease aphid numbers especially early in the year, as aphids not protected by the ear are easily dislodged from wheat plants (Watson & Carter 1983). Similar results were shown by our microcosm experiment (**Chapter 4**), as the risk for aphids was more than two-times higher being dislodged by rain from shoots than from ears. Increased aphid availability on the soil surface might trigger ground dwelling predator effects (Winder *et al.* 1994). In fact, we found significantly more generalist predators which consumed aphids after an artificially heavy rain shower than without rain (**Chapter 4**). This suggests possible synergistic effects of abiotic factors and ground dwelling predators in the field, potentially enhancing biological control.

Predator identity: Our field experiments documented the importance of single predator species for aphid control. Especially the staphylinid *Philonthus fuscipennis* presumably contributed to aphid suppression in both field experiments (**Chapter 2**, **Chapter 3**); also in previous studies this species has been shown to be an important aphid predator (Sopp & Wratten 1986; Dennis & Wratten 1991). In the mesocosm experiment, this species also consumed aphids at high rates, together with two spiders and a carabid beetle (**Chapter 5**). The results suggest that high abundances of early season predators contributes to aphid control (**Chapter 3**), however, interestingly a predator species which was abundant early in the year, thereby decreasing aphid populations, did not affect aphid populations if abundant

later in the year (**Chapter 2**). Further, different generalist predator species controlled aphid populations in different fields to a similar extent, suggesting that some species within the generalist predator guild can provide the same ecosystem service. These results indicate that both the temporal as well as the spatial scale need to be considered for optimizing the effectiveness of herbivore control by generalist predator species. The necessity to analyse trophic interactions at multiple spatial and temporal scales has been stressed before (Kareiva 1990; Pimm 1991; Rosenzweig 1995; Holt 1996; Roland & Taylor 1997; Wiens *et al.* 1997; Menalled *et al.* 2003; Tscharntke & Brandl 2004), especially for agricultural landscapes, where management practices such as annual harvesting and soil cultivation change animal communities regularly (Thies *et al.* 2005), thereby modifying biological control (Kruess & Tscharntke 1994; Thies & Tscharntke 1999; Östman *et al.* 2001; Östman & Ives 2003; Thies *et al.* 2003).

Scavenging: Generalist predators are not only confronted with a wide range of living prey but also with dead prey. In the field arthropods die because of parasitism, disease, adverse weather conditions, starvation or naturally, thereby providing food for potential scavengers (Putman 1983; Winder *et al.* 1994; Sunderland 1996). Scavenging is assumed to be widespread in generalist predators (Sunderland 1996), but information from the field is difficult to obtain. In our experimental winter wheat system generalist predators consumed dead aphids at high rates, and scavenging was found in carabids, staphylinids and spiders (**Chapter 5**). Carrion represents an easily available source of energy (Lang & Gsödl 2001), and could therefore disrupt biological control if carrion availability is high in the field and generalists prefer dead over living prey. High abundance of dead aphids on the soil surface was reported by Sopp *et al.* (1987) and preferences for dead over live prey in generalist predators were shown for aphid prey (Foltan *et al.* 2005) and slug prey (Langan *et al.* 2001; Mair & Port 2001). Also, results of the microcosm experiment suggest that due to rain high proportions of dead aphids were available and consumed by ground dwelling predators, possibly impeding control effects on aphid populations (**Chapter 4**). However, it has been shown that increased foraging activity in response to highly available prey may initiate opportunistic predation and possibly suppression of a second prey (Prasad & Snyder 2006a).

Certainly, more studies are necessary to evaluate the role of scavenging in generalist predator – prey interactions; the finding that also spiders (tetragnathids) may consume dead aphid prey suggests that scavenging is more widespread than previously assumed and needs further attention.

7.2. MOLECULAR TECHNIQUES

DNA-based techniques are highly sensitive in detecting prey DNA in predator guts. A series of laboratory feeding experiments using various predator-prey systems have been performed in the last decade (reviewed in Symondson 2002; Sheppard & Harwood 2005; Gariepy *et al.* 2007), investigating prey DNA detection in guts of arthropod predators. Recently, this method has also been used to screen predators caught in the field (Agusti *et al.*, 2003; Harper *et al.*, 2005; Greenstone *et al.*, 2007; Juen & Traugott, 2007), but caution is needed in interpreting field results.

As DNA is both spontaneously and enzymatically decaying, prey DNA detection is temporally restricted. This has been shown by several authors and depends on a number of factors modifying DNA breakdown.

Fragment length has been shown before to affect DNA detectability, with shorter fragments being longer detectable than the larger fragments. It has been suggested that these differences could be used to determine the time when predation occurred (Hoogendoorn & Heimpel 2001). We showed, however, that fragments differing by about 100 bp yielded similar detectability rates (**Chapter 6**), and these results confirm previous studies also showing no differences in detection rates between fragments differing by one to several hundred base pairs (Chen *et al.* 2000; Juen & Traugott 2005, 2006, 2007). However, using a set of primers amplifying fragments of distinct differences in amplicon size may allow estimating the time since feeding, but the resolution of the time scale derived by the amplification of such fragments is suspected to be restricted. Nevertheless, ecologically relevant questions can be answered, for example differentiation between night and diurnal predator activity or temporal niche differentiation of predators.

Ambient temperature was shown to affect prey DNA detectability, but significant differences were only present at temperatures above 20 °C (**Chapter 6**), corresponding to the results of D Read et al. (unpublished data) who found also only temperatures above 20 ° to affect detection times of nematode DNA in the gut of collembolans. If it is confirmed that lower temperatures (< 20 °C) do not affect DNA detection rates, temperature effects presumably can be neglected in systems where ambient temperatures fluctuate only within a small range, e.g. in soil-dwelling predators (Juen & Traugott, 2007) or epigeic predators hunting under a dense plant canopy.

An important factor affecting detection rates is *species identity*. Previous studies showed significant differences of mean detection rates of prey DNA between different predator taxa, most significantly between predators differing in their feeding mode. It has been suggested that detection half-lives may differ primarily between taxonomically and physiologically different species (Greenstone *et al.* 2007). However, we showed significant differences in detection rates between two closely related predator species with similar feeding and hunting mode (**Chapter 6**), underscoring the insufficiency of solely investigating prey DNA detectability in predator taxa differing in their feeding modes. Therefore, due to possible differences between detection rates of prey DNA in different predator taxa we did not rank the predator species from our mesocosm experiment for their possible aphid control efficiency, but rather compared detection rates of live and dead aphids within predator species (**Chapter 5**).

Scavenging potentially bears a risk in false interpretation of predator-prey interactions in the field. A wide range of generalist predators scavenged on dead aphids including a spider species (**Chapter 5**). As DNA-based techniques do not differentiate between living and dead prey positive tested predators only indicate consumption of the prey. In terms of predators' biological control efficiency one is interested in predation, demanding for additional data from the field such as carrion availability (Sunderland 1996; Foltan *et al.* 2005). On the other hand the sensitivity of the DNA-based technique opened up a method to investigate scavenging of a predator guild by simultaneously detecting live and dead prey DNA in generalist predator guts (**Chapter 5**).

Applying DNA-based techniques in field studies deserves highly specific PCR systems. Specificity of primers being used in laboratory feeding experiments may not be specific enough for screening field caught predators. Especially generalist predators feed on a wide range of prey, and due to scavenging and intraguild predation further species contribute to food remains in predator guts. Therefore, primers ideally have to be tested against all these possible 'prey' species to ensure not to gain false positives. To investigate factors modifying predator-prey interactions simplified systems (meso- or microcosms) are helpful by reducing the spectrum of potential errors affecting prey DNA detectability. We successfully employed DNA-based gut content analyses in a microcosm experiment and documented that abiotic factors modify aphid consumption by ground dwelling predators, and also identified scavenging in generalist predators. Therefore, DNA-based techniques hold more than the possibility to investigate trophic links in the field, it also opens up new possibilities in investigating ecological processes modulating predator-prey interactions in simplified model systems.

7.3. CONCLUSIONS

The present work demonstrated the ability of generalist predators to significantly suppress aphid populations in winter wheat fields (**Chapter 2**, **Chapter 3**). Large scale mulch addition triggered a trophic cascade enhancing the density of microbi-detritivores and predators, thereby increasing herbivore suppression (**Chapter 2**), with single predator species dominating aphid control. Presence of belowground alternative prey did not disrupt herbivore control, as collembolans and aphids both contributed to predator diets (**Chapter 3**), and increasing aphid densities induced a shift of predators from belowground to herbivore prey (**Chapter 3**). These results indicate that detrital subsidies can foster biological control, but also call for further field studies taking into account temporal variations and spatial heterogeneity. Abiotic factors also modulate predatory effects on prey populations. Rain significantly increased consumption rates of aphids by ground dwelling predators (**Chapter 4**), but did not significantly increase aphid suppression. Presumably, predators fed preferentially on dead aphids, and in fact scavenging has been demonstrated for a wide range

of generalist predators (**Chapter 5**). This suggests that abiotic and biotic factors modulate predator-prey interactions, thereby complicating predictions on the effectiveness of biological control. Molecular techniques proved to be very useful in investigating factors affecting herbivore control (**Chapter 4**, **Chapter 5**), and are recommended for extensive use in micro- and mesocosm studies. Also, in field studies DNA-based techniques will contribute to our knowledge of trophic links, but processes, such as DNA decay in the gut of predators and the mode of feeding of predators, need further attention (**Chapter 6**). Especially differences in detection rates of prey DNA between closely related species call for further analyses; this knowledge is crucial for evaluating the potential of individual species for biological control.

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe.

Ich habe noch keinen Promotionsversuch unternommen

Darmstadt, den 16.11.2007 Karsten von Berg