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Spatial distribution of soil insects in grassland

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SPATIAL DISTRIBUTION OF SOIL INSECTS IN GRASSLAND

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by

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Spatial distribution of soil insects in grassland

Abstract

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Recently, there has been interest in the study of soil biodiversity and its functional role in agricultural grassland due to the need to develop management strategies for soil biota to improve ecosystem self-regulation. In the UK, grasslands provide a habitat for several subterranean insect taxa some of which are pests - leatherjackets (larvae of Tipulidae: Diptera) and wireworms (larvae of Elateridae:Coleoptera). To provide an appropriate method of pest management, a systematic sampling of agricultural grassland over different sampling scales is crucial in order to understand their population distribution and distribution patterns. The spatial distribution and distribution patterns of soil insects were investigated in agricultural grassland over three sampling scales (farmlet, field and core). The effect of spatial, biotic and scale variables and their combinations have been assessed. In addition, a new molecular method has been developed to identify grassland leatherjackets using universal DNA primers.

Soil core samples were collected from 19 grass fields divided into 3 farmlets in the Farm Platform at Rothamsted Research, North Wyke. The abundance of taxa and presence: absence data were analysed over the three sampling scales using variance/mean ratios, non-metric multidimensional scaling to visualise association between individual taxa, and deviance partitioning to determine the effect of spatial, space and biotic variables on species distribution. The spatial distribution of most taxa varied between sampling scales from randomness at the core scale to aggregation at the field and farm scales, though some taxa were aggregated at

nearly all scales. Using deviance partitioning our results support previous reports and suggest that scale is the most important factor influencing taxa distribution in comparison with biotic and space variables which had a minor impact. This could explain the variation between taxa which might be due to the interactions between specific environmental variables and sampling scale, biotic and other unknown factors and species biology. DNA barcoding of *Tipula* spp. using universal DNA primers showed that leatherjackets in grassland are dominated by *Tipula paludosa* with *T*. *oleraca* only found in small numbers. There were also some species of *T*. *paludosa* with high genetic variability (P>3%) within the species indicating the possibility of other cryptic species close to *T. paludosa.*

List of abbreviations

Abbreviations name	Description
ANOSIM	Analysis of similarities
BLAST	Basic local alignment search tool
BOLD	Barcode of life data base
BSA	Bovine serum albumin
COI	Cytochrome c oxidase subunit 1
DM	Dry Matter
DNA	Dioxy Nucleic Acid
dNTPs	DeoxyribonucleosideTriphosphate Solutions
EPN	Entomo Pathogenic Nematodes
ESRI	Environmental Systems Research Institute
EXO	Exonuclease
FAO	Food Agriculture Organization
FP	Forward primer
GIS	Geographic information system
GPS	Global Positioning System
Ha^{-1}	Hectare
HN	Haplotype number
ITS	internal transcribed spacer
MEGA	Molecular evolutionary genetics analysis
NBN	National Biodiversity Network
NJ	Neighbour joining
NMDS	Non-metric Multi Dimensional Scaling
ODCB	OrthoDiChloro Benzene

PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PTA	Partial Triadic Analysis
RFLP	Restriction Fragment Length Polymorphism
RNP	Relative Net Precision
RP	Reverse primer
SADIE	Spatial Analysis by Distance Indices
SAP	Shrimp Alkaline Phosphatase
TIV	Tipula Iridescent Virus
TNPV	Tipula Nuclear Polyhydrosis Virus
UK	United kingdom
USA	United States of America
µl	Microliter

List of contents

List of tables

List of figures

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Author's Declaration

At no time during the registration for the degree of Research Master (ResM) has the author been registered for any other University award without prior agreement of the Graduate Committee. This study was financed with the aid of Kurdish Regional Government (KRG) and The Ministry of Higher Education and Scientific Research (MOHESR) of Kurdistan, Iraq.

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Chapter 1

General Introduction and literature review

Chapter 1: General Introduction and literature review

1.1 Introduction

Grasslands comprise the primary vegetation in many environments. Natural grasslands cover millions of hectares across the world, supporting a wide range of wildlife (Klein *et al*., 2007). They are home ground for various insects and may hold an outstanding species rich community. For instance, old temperate grassland may hold more than 1500 species and their communities change depending on climate, soil quality, and management practices (Resh & Cardé, 2009). Most of those species rely on soil for at least one part of their Lifecycle, among them the insects of the orders Diptera, Lepidoptera and Coleoptera, some of which are economically important particularly due to their immature stages (larvae) and are considered to be pests of agricultural crops (Wallwork, 1976). Their ability to burrow in the soil can protect them from long or short term unfavourable environmental conditions such as high or low temperature, wind, evaporation, light and dryness (King, 1939).

In the UK, grassland comprises a predominant part of the agricultural land in the country and is the basis of livestock production (FAO, 2008). 6.4% of the total agricultural area comprises newly sown grasslands (less than five years age), 32.3% consists of grasslands of more than five years age known as old or permanent grassland, and 31% of total agricultural land consists of crops. Others such as rough grazing and common grazing cover 30.3% of the total agricultural area (Table 1.1) (FAO, 2008).

Table 1.1 Areas of grassland and other agricultural land use in the UK (as '000 ha) [based on 2006 data] (FAO, 2008).

Grassland and other agricultural land	England	Wales	Scotland	N.Ireland	All UK
Crops and tillage	3,840	66	1,566	187	5,659
Grass < 5 years old	590	115	325	136	1,166
Grass > 5 years old	3,330	982	910	676	5,898
Rough grazing in sole rights	640	221	3,340	149	4,350
Common rough grazing	395	180	598	29	1,202
Total agricultural land*	8,795	1,564	6,739	1,177	18,275

*excludes woodland on farms and set-aside land

The majority of Grasslands in the UK belong to three major types of semi-natural grassland depending on the quality of the soil (Duffey & Station, 1974) which are:

1- Calcareous grassland: made of soils that contain different types of calcareous parent materials. They are of high pH and organic matter.
2- Neutral: these are clay or loamy soils.
3- Acidic: They are the most common types of semi natural grasslands in the UK.

1.2 Insect taxa in grassland soils

Benefer *et al*. (2012) has indicated that in the UK, the subterranean insects in grassland are dominated by larvae: wireworms (*Agriotes sp*.), leatherjackets (*Tipula sp*.), bibionids (Bibionidae) and larvae of (Sciaridae). Other insects occur in grassland soils such as the larvae of *Agrotis spp.* known as cutworms

(Williamson & Potier, 1997). Some are economically important and considered to be pests to agriculture such as the larvae of *Agriotes spp*. (wireworms) which have been reported to be the worst pests of arable lands (Buckle, 1923), and the larvae of *Tipula* spp. (leatherjackets) which are the most serious pest of grasslands (Blackshaw, 1985; Blackshaw, 2006; Buckle, 1923; French, 1970; Peck, Olmstead & Petersen, 2010; White & French, 1968). Others are considered as sporadic pests but can cause significant damage in grasslands when they appear in large populations such as the larvae of Bibionid fly (D' Arcy – Burt, 1987) and several species of lepidopteran larvae (Curry, 1994).

1.2.1 Tipulidae (Leatherjackets)

1.2.1.1 Life cycle

Among leatherjacket (European crane fly larvae) species, two species *Tipula paludosa* Meigen and *T. oleracea* L. are known to be pests of agricultural crops particularly in grassland and spring cereals (Blackshaw & Coll, 1999; Peck & Olmstead, 2009). *Tipula paludosa* is a prominent univoltine soil insect (Blackshaw & Coll, 1999; Milne, Laughlin & Coggins, 1965). During August and September, adults start to appear after their emergence from pupae near the surface of the soil. After mating, females lay their eggs in grass on the surface of the soil. The majority of the eggs are laid before dawn and entirely in a period of 32 hours following emergence (Coulson, 1962; Milne, Laughlin & Coggins, 1965). Eggs hatch in about 15 to 21 days and larvae continue to feed and increase in size in the soil during winter and spring. Moulting starts for the first and second instars at the end of September and continues through November until April for the third and fourth instars respectively (Blackshaw & Moore, 2012). Before the end of

June, they stop feeding and the fourth larval instar goes through the process of a pre-pupal stage and then a pupa which builds its pathway toward the soil surface to enable adults to emerge (Johnson & Murray, 2008; Milne, Laughlin & Coggins, 1965). They stay in the pupal stage for about two weeks and the adults start to appear during several weeks and they reach a peak number from mid-August to mid-September in the UK. The total duration of the larval stage is at least nine months in the soil and the insect has one generation per year (Rennie, 1917). A study by Coulson (1962) showed that in the UK, in northern England at Moor House nature reserve, the majority of them appeared six weeks before their occurrence at Rothamsted in the south. Other studies by Blackshaw (1983a) showed that in Northern Ireland the highest numbers of *Tipula paludosa* appeared in mid-August to mid-September.

Blackshaw (1983b) used water traps to catch adults of *T. oleracea* and found that their total number is lower than *T. paludosa*; however, further studies by Coll *et al*. (1993) showed that there were some unexpected and severe crop losses by *T. oleracea* in winter cereals following oil seed rape. In the northeast United States, a study by Peck and Olmstead (2009) showed that from 2004 to 2006 the appearance of *T. paludosa* was recorded in four counties whereas *T. oleracea was* recorded in 12 counties. The same study found that the occurrence of *T. oleracea* in western New York is six times greater than *T. paludosa*. Studies showed that the appearance of *T. oleracea* varies according to climatic variations, for example in the UK two obvious flight periods are noticed, one at the end of April till June and the other one from September to October (Miles, 1921). Dobson

(1974) recorded *T. oleracea* in Craibstone in Aberdeen from May to the end of October.

T. oleracea is a multivoltine but happens to have two generations in its normal range and does not spend as much time in the soil as *T. paludosa*, therefore, the typical lifecycle for leatherjackets is not applicable for *T. oleracea* (Blackshaw, 1999). In addition, most of the laboratory experiments about leatherjackets have used *T. oleracea* in comparison with *T. paludosa* because managing *T. oleracea* in the lab is easier than *T. paludosa* (e.g.Carter, 1975; Ehlers, Wulff & Peters, 1997; Wiegers, Dullemans & Wijbenga, 1992). *Tipula paludosa* is considered to be more arduous to culture in the lab in comparison with *T. oleracea* due to the long period of the larval stage in the soil. The growth of *T. paludosa* larvae can be followed because larval instars can be differentiated depending on head capsule width and posterior spiracular disk diameter; a similar method can be used to determine gender in the 4th instar (Blackshaw & Moore, 1984). Pinchin and Anderson (1936) found that the activity of *T. paludosa* varies in comparison with *T. oleracea*; for *T. paludosa* the activity appeared at dawn whereas for *T. oleracea* most activity appeared after dusk and repeatedly after midnight.

1.2.1.2 Pest species

The family Tipulidae *sensu stricto* includes 4276 species and subspecies which are distributed globally (Oosterbroek, 2013). Smith (1989) reported that In the UK, Tipulidae comprises the biggest dipteran family and contains 303 species. A recent report by the National Biodiversity Network (NBN, 2013) showed that the total number of recorded species of Tipulidae in the UK based on summer 2006

data is 340 species from 13828 sites (Table 1.2) including England, Wales and Scotland with a small number of records from Ireland. Recording densities varied but were much higher in parts of northern and south-eastern England in particular for *T. paludosa* (Figure 1.1).

Table 1.2 Crane fly (Diptera; Tipuloidea) records in Britain in 2006 (NBN, 2012).

Number of records	109452
Number of recorded sites	13828
Number of recorded samples	34520
Number of recorded species	340

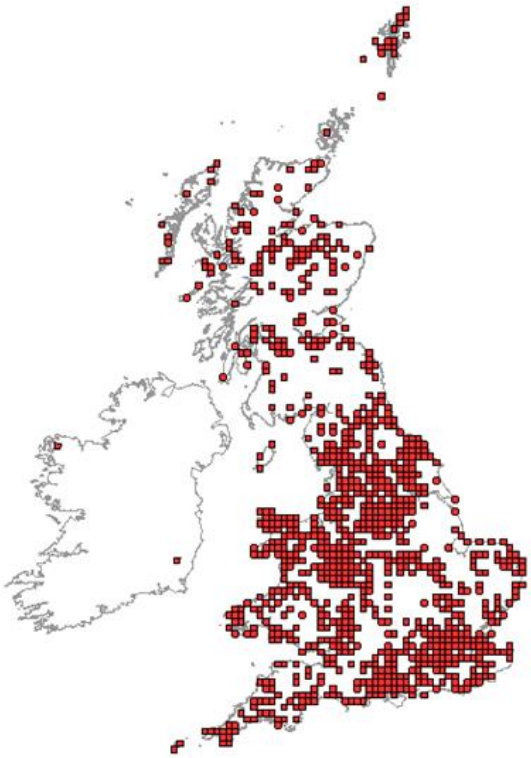


Figure 1.1 10km squares with records of *Tipula paludosa* in Great Britain and Ireland (NBN, 2013).

In spite of the huge number of recorded species, only a small number of species are known to be pests of agricultural and horticultural crops (see Blackshaw & Coll, 1999). For instance, larvae of *Tipula* (*Yamatotipula*) *aino* are of economic importance damaging rice seedlings (de Jong *et al*., 2008). Larvae of *T. varripennis* Meig, *T. vernalis* Meig and *T. subnodicornis* Zetterstedt (Blackshaw, 1991) are usually considered as minor pests whereas for *T. paludosa* known as the marsh fly and *T. oleracea* L. known as the cabbage crane fly, their larvae are known to be major pests of agriculture (Blackshaw, 1991; Peck & Olmstead, 2009). Previous studies indicate that regarding their impact as agricultural pests *T. paludosa* is the most important of the two species (Brownbridge & Selman, 1989; Deleporte, 1981). Because of the difficulty in achieving a precise identification, most researchers consider leatherjackets as the larvae of *Tipula* species (Benefer *et al*., 2010; Blackshaw & Newbold, 1987; Tucker & Cutler, 1979). Thus, a research challenge is to identify these two cryptic leatherjackets.

1.2.1.3 Dispersal

Leatherjackets have been documented in many parts of the UK including England, Scotland, Northern Ireland (Blackshaw, 1983a; Grennan, 1966; Mayor & Davies, 1976; NBN, 2012b) and other European countries such as the Netherlands and Norway (Blackshaw, 1999). Benefer *et al.* (2010) reported *T. paludosa* in six grass fields from Seale Hayne Farm, South Devon, UK in 2008. In the United States, *T. paludosa* and *T. oleracea* were reported from New York City as pests of turf and pasture grasses between 2004 and 2006 (Peck, Hoebeke & Klass, 2006; Peck & Olmstead, 2009). Dobrotworsky (1968) reported 19 genera of the subfamily Tipulinae from Australia. Byers & Arnaud (2011) reported *T.*

oleracea L. in West–Central California. In a survey to determine the distribution of leatherjackets in northern Britain, Humphreys *et al* (1993) used isoelectric focusing to differentiate between the larvae of *T. paludosa* and *T. oleracea* and their results showed that the larvae of *T. paludosa* was the most prominent species in comparison with *T. oleracea* which was not found frequently in agricultural grasslands. It is not known if this result is typical of grasslands elsewhere.

1.2.1.4 Damage to crops

The larvae of *Tipula spp.* known as leatherjackets are considered to be substantial pests of agriculture particularly organic crops (Chandler, 1998). Blackshaw (1984) investigated the damage in grassland caused by leatherjackets and found that 125,000 leatherjackets ha^{-1} in March led to a significant loss of 50 kg herbage dry matter (DM) ha^{-1} by mid-May. Other studies, such as White and French (1968), showed that spring cereals are exposed to damage by leatherjackets and they found that leatherjackets can cause significant yield loss through their feeding on the roots of those crops and sporadically on the leaves or grains; affecting plant germination. Additionally, the symptoms of the damage can be noticed through the occurrence of bare patches in the field. The damage by leatherjackets has also been investigated on winter cereals in northern Britain by Coll *et al.* (1993) and suggests that leatherjackets can cause significant damage to winter cereals through decreasing crop yield. Further studies by Blackshaw and Coll (1999) reported the damage by leatherjackets on a wide range of Non-Graminacae and suggests that a variety of crops can be damaged by leatherjackets such as sugar beet, sweet-corn (Emmet, 1992) berries, tobacco,

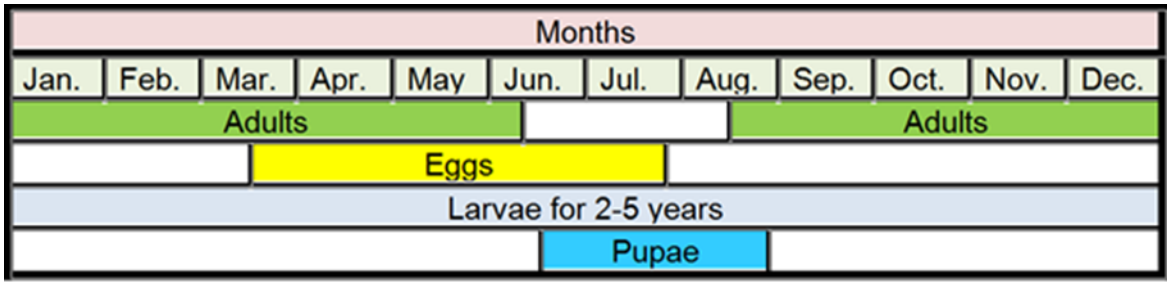
various vegetables and ornamentals (Campbell, 1975). This indicates that leatherjackets are polyphagous insects in their feeding (Blackshaw, 1999).

1.2.2 Elateridae (Wireworms)

1.2.2.1 Life cycle

Shortly after mating, female click beetles start laying their eggs under the surface of the soil in particular grassy areas. The number of eggs varies from 50-350 eggs or more and eggs are laid either individually or in groups (Andrews *et al*., 2008). Parker and Howard (2001) reported that in the UK, the eggs of *Agriotes spp.* hatch in about four to six weeks according to variability in temperature. The first larval instar starts to appear in June and July and moves deeper into the soil (Roberts, 1921). In the UK, they spend more than four years in the soil prior to pupation (Miles, 1942). The pupal stage lasts about 21 days and adults (male and female) start to appear at the end of spring and through the summer (Table1. 3) (Berry *et al.,* 1998).

Figure 1.2 Wireworm life cycle. Adapted from Berry *et al*., (1998).



1.2.2.2 Pest species

One of the most substantial groups of pests in agricultural soils are the larvae of click beetles known as wireworms (Coleoptera: Elateridae) which are considered to be harmful globally (Tóth *et al*., 2003). They include different species and

families; e.g. Branch and Bousquet (1991) reported that the click beetle family (Elateridae) includes 369 species and subspecies in Canada and Alaska. They live in different habitats such as foliage and flowers, under bark and in the soil. In addition, that study also reported that those species whose larvae live in the soil can cause significant damage to crops. In central Europe, more than 20 species of click beetles within the genus *Agriotes* are found in arable lands. These species vary in their habitat and their damage to agriculture (Staudacher et al., 2011b). In the UK, three species of click beetles belonging to the genus *Agriotes spp. - A. obscurus, A. sputator* and *A*. *lineatus* - are considered as a complex of pests primarily due to difficulty in their morphological identification (Benefer, 2012). Another study by Benefer *et al* 2010 showed that in South Devon, UK, among the three species of *Agriotes spp*, *A*. *lineatus* were the most numerous species with their larvae associated with grassland soils followed by *A. sputator* and *A . obscurus*.

1.2.2.3 Dispersal

Wireworms are widely distributed insects in many parts of the world. *Agriotes spp.* is found in Europe, Asia, and North America. Other genera such as *Ctenicera* (= *Corymbits*), *Athous* (garden wireworms), *Ectinus*, *Melanotus*, *Limonius* and *Lacon* (tropical wireworms) are found in Europe, UK, USA and Asia including Japan (NBN, 2012b). In a wider study on European click beetles, Tóth *et al* (2003) used pheromone traps to investigate the dispersal of click beetle species and the study concluded that *A. lineatus* is the predominant species of wireworm in most countries including the United Kingdom, Germany, Austria, Switzerland, Italy, Slovenia, Croatia, Romania, Bulgaria, Greece, and Hungary. However, no *A.*

lineatus were detected in Veneto, Italy. Alternatively, in Portugal, *A*. *proximus* were detected. In the UK, the genus *Agriotes* is the most abundant click beetle species distributed widely in grassland soils (Benefer, 2012). The dispersal ability of wireworm species within the genus *Agriotes* may vary in comparison with adults and the presence of adults are not necessarily indicative of the presence of larvae (Benefer, 2012; Blackshaw & Hicks, 2012). Additionally, Blackshaw *et al* (2009) used sex pheromone traps to investigate the populations of wireworms within the genus *Agriotes* and presented evidence that sex pheromone trap counts cannot directly be compared for different species. They reported greater catches for the species *A. lineatus, A. obscurus* and *A. sputator* respectively even though numbers released were the same (Blackshaw *et al* 2009).

1.2.2.4 Damage to crops

A variety of crops are known to be damaged by wireworms; e.g. Lane (1931) reported that the Great Basin wireworms can cause considerable damage to both winter and spring cereals and cause farmers of the Pacific North West to lose millions of dollars each year. A study by Gibson (1939) showed that in Yakima Valley, (Washington State) wireworms caused significant damage in potato fields. Moreover, further studies have investigated the damage on potatoes caused by wireworms (e.g.Chalfant et al., 1990; Jansson & Lecrone, 1991; Pantoja, Hagerty & Emmert, 2010b; Pantoja, Hagerty & Emmert, 2010a; Parker & Howard, 2001). Other crops are known to be damaged by wireworms (Table1.3) Indicating that they are polyphagous in their feeding habits.

Table 1.3 Damage to crop varieties by wireworms.

Name of crops damaged by wireworms	Author
Corn	(Cheshire *et al.*, 1987)
Corn and Cotton	(Gibson, 1916)
Straw berries and wheat	(Vernon, Kabaluk & Behringer, 2000)

Apart from feeding on crops, wireworms are thought to feed on other food sources such as weeds, soil organic matter and animal prey; however, the study on feeding ecology by wireworms under natural conditions is poor (Traugott *et al*., 2008). Recently, Devetak & Arnett, (2010) stated that the click beetle larvae of the subfamily Cardophorinae may be phytophagous, saprophagous or predacious. It was found for the first time that the Cardophorinae larvae are predacious on the larvae of antlions (Myrmeleontidae) (Figure 1.2) (Devetak & Arnett, 2010).

Figure 1.3 Larva of the elaterid subfamily Cardiophorinae with its prey, a third-instar larva of the Antlion *Myrmeleon immaculatus* (Devetak & Arnett, 2010).

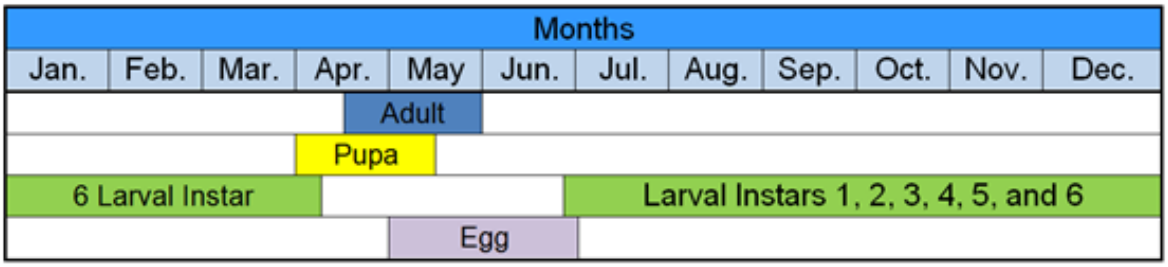
1.2.3 Bibionidae (Bibionids)

1.2.3.1 Life cycle

D'Arcy-Burt (1987) reported that in Northern Ireland, in most years, bibionid species appear from April to November, but the appearance is more during the months of May and June. Shortly after mating, females penetrate the soil to a depth of 3-5 cm or more, using their strong front tibiae with tarsi folded back. Each female lays a mass of 200-300 cylindrical eggs in a small cell made by the female and they die outside the cell afterwards. Males do not survive, and die shortly after mating (Freeman & Lane, 1985). The larvae exist in damp soil, vegetable debris, roots of grass and other plants, such as seedlings, and manure.

They start feeding on different crops and damaging them, such as cereals, vegetable varieties, as well as ornamentals and grass (Brown *et al*., 2009; Freeman & Lane, 1985). Larvae of *Bibio* and *Dilophus* are considered to be pests in both agricultural and urban environments, through damaging live plant structures in the soil (D' Arcy - Burt & Blackshaw, 1991). The fully grown larvae reach 20-24 mm in the larger species. Most of the species are univoltine, but some have two flight periods per year (D'Arcy-Burt, 1987; Freeman & Lane, 1985). The larvae pupate in the soil, forming chambers and the adult flies start to appear three weeks later, digging their way out of the soil (Freeman & Lane, 1985). The suggested life cycle of *Bibio johannis* in the UK, based on the flight periods of the species (D'Arcy-Burt, 1987), is shown in figure 1.4.

Figure 1.4 Life Cycle of *Bibio johannis.* Adapted from D'Arcy-Burt, (1987).



1.2.3.2 Pest species

Many species of bibionid flies have been found belonging to the family Bibionidae in different environments such as grasslands, forests, and agricultural ecosystems (Skartveit & Kaplan, 1996). Hardy and Takahashi (1960) reported that in Japan, four species of Bibionidae including *Penthetria motschulskii*, *Bibio flavihalter*, *B. gracilipalpus* and *B. metaclavipes* occur between fall and early winter whereas *B. ryukyuensis* emerges from January to March, and *P. velutina*

Loew appears throughout the year. Hardy *et al*. (1969) showed that in the Philippines, 27 species of Bibionidae are known. In the Nearctic region, among 75 species of Bibionidae, 21 are known to appear in California (Hardy, 1961). Li and Yang (2010) described two new species of Bibionidae belong to the genus *Penthetria* Meigen in Yunnan, China for the first time which were *P. medialis, P. zhongdianensis.*

Despite this large number of Bibionidae species, they are not considered as major pests and the number of published papers in this area is rather scarce (D' Arcy - Burt & Blackshaw, 1991). In The UK, the most common species of grassland Bibionidae have been investigated by D'Arcy-Burt (1987) in Ireland and the study showed that the majority of the species belong to the genus *Bibio*, followed by *Dilophus*. However, in other parts of the UK, such as Devon, studies on the biology and ecology of Bibionidae are still scarce.

1.2.3.3 Dispersal

The family Bibionidae has a global dispersion and comprises about 700 species belonging to 8 genera which vary in their species pattern (Fitzgerald, 2005). For example, in the United States, *Bibio* has 32 species, in Mexico 15 species, in Central America 6 species, with 6 in South America. Whereas 51 species belonging to the genus *Plecia* have been reported in South America, only two species are found in the U.S (Fitzgerald, 2005). The genera *Bibio*, *Dilophus*, and *Plecia* are virtually worldwide distributed and have been reported from every continent excluding Antarctica (Fitzgerald, 2005).

Bibionid larvae have been reported as pests of grasslands particularly turf grasses in North America, Europe and Asia (Potter, 1998). In the UK, D'Arcy-Burt

(1987) studied the distribution of Bibionidae in Irish grasslands and recorded 9 species belonging to two genera (7 *Bibio* and 2 *Dilophus*) using yellow water traps. Recent studies by Benefer et al. (2010) reported the larvae of Bibionidae in grassland soils in South Devon, UK being either *B. johannis* L. or *D. febrilis* L. .

1.2.3.4 Damage to crops

Bibionidae larvae are known to damage a variety of crops. In grasslands, they are associated with stem bases of grasses but other parts of plants are damaged such as leaves. The larvae have the ability to remove the soil around the plant leaving it exposed to other damage such as frost and dehydration (D' Arcy - Burt & Blackshaw, 1991). Similar damage can be caused by leatherjackets that sometimes cannot be distinguished from them (D' Arcy - Burt & Blackshaw, 1991). D'Arcy-Burt (1987) concluded that in the UK, bibionids appear as sporadic pests and can cause damage to grasses and cereals as well as some arable and horticultural crops. Other crops are also known to be damaged by bibionids such as peas and celery (Freeman & Lane, 1985) and they are considered as phyto-saprophagous insects; feeding on both dead and living plant tissues (Pecina, 1982).

1.2.4 Noctuidae (Cutworms)

1.2.4.1 Life cycle

Cutworms (Lepidoptera: Noctuidae) have different common names such as greasy, variegated, glassy and spotted cutworm. However their habits are similar (Walton, 1920). They are nocturnal insects. Females lay about 200-500 eggs either individually or in groups in grassy fields. The larvae emerge from the egg in

September several days after the eggs are laid and start feeding on grasses and other plants until the weather starts to be cold in winter (Walton, 1920). The species *Agrotis ipsilon* (Hufnagel), known as black cutworm, goes through six larval instars prior to pupation (Table1.4). About 15 days after pupating adults start to appear, mate and start their life cycle again. The insect is multivoltine and has three generations per year (Delahaut, 2007) whereas others such as *Abagrotis orbis* and *Agrotis vetusta* are univoltine and they start their mating and egg laying during the fall, followed by overwintering as small larvae (Wright *et al*., 2010).

Table 1.4 The activity of black cutworm under different life stages (Delahaut, 2007).

Life stage of black cut worm	Activity
Intensive moth capture	Egg laying
Egg hatch	------
1^{st} – 3^{rd} Instar	Leaf feeding
4^{th} Instar	Cutting begins
5^{th} Instar	Cutting
6^{th} Instar	Cutting slows
Pupa to moth	Cutting stops

1.2.4.2 Pest species

A wide range of cutworm species is known as pests of agriculture. Lowery and Mostafa (2010) reported 18 species of cutworms as pests to grape vines in the Okanagan Valley, British Columbia, Canada from 2004 - 2008. Ten species were recorded belonging to the genera *Abagrotis* and *Euxoa.* Wright *et al*. (2010) reported 25 species of cutworms in vineyards in south central Washington-USA, about 75% of the cutworms being *A. orbis* (Grote 1876) and 19% were *A. vetusta*

Walker. Others such as the spotted cutworm, *Xestia c-nigrum* L. and the red black cutworm, *Euxoa ochrogaster* (Guenee) were formerly considered as considerable pests to grapes in Washington. The black cutworm *A. ipsilon* is one of the major pests of agriculture. The larvae damage vegetable plants or new grown seedlings and have been considered as a serious pest of vegetables from 19 countries on 5 continents (Rings, Arnold & Johnson, 1975). The species *Agrotis segutum* Schiff is also a wide distributed pest of sporadic importance on a wide range of crops and the damage can occasionally be devastating (Hill, 2008). It is considered as the major pest species in the UK and northern Europe (Alford, 2000; Esbjerg & Lauritzen, 2010) which is polyphagous and damages the seedlings of many crops, and many vegetables and root crops (Hill, 2008).

1.2.4.3 Dispersal

Cutworms are widely distributed around the world with different environments (Ortega, 1987). The black cutworm is found in North America, Europe, Asia, Africa, and elsewhere (Capinera, 2008). Wright *et al*. (2010) reported the larvae of cutworms in South Central Washington Vineyards during spring. In Manitoba, Canada, several species including *E. ochrogaster* (Red backed cutworm), *E. messoria* (Harr.) (Dark sided cutworm) and *Feltia jaculifera* (Gn.) are distributed in the country in tillage fields and are considered as pests to those fields (Turnock, Timlick & Palaniswamy, 1993). Further Studies in Israel investigated the occurrence of different species of Noctuinae and recorded 9 new species for the first time which were *E. conspicua*, *E. hering*, *A. psammocharis*, *Agrotis* (*Powellinia*) *boetica*, *Pachyagrotis tischendorfi*, *Dichagyris melanuroides*, *Dichagyris amoena*, *Noctua tertia* and *Noctua interjecta* (Kravchenko *et a*l., 2006).

Moreover, in grasslands, Kruger (2005) reported three genera of Noctuidae being *Malotia, Diargyria* and *Paradigma* in alpine grasslands in Lesotho, Africa. However, the lepidopteran populations in grasslands are low in general and less than 10 m^{-2} (Curry, 1994).

1.2.4.4 Damage to crops

Cutworms are considered as general soil pests. They feed on plants at soil level and are able to damage new established grasslands (Curry, 1994). They are nocturnal insects and feed on plants at night. Some of the species may feed on plants during the day but they stay under the soil surface at that time (Ortega, 1987). The black cutworm (*A*. *ipsilon*) is one of the most damaging cutworms that attacks turf grasses. It is the main pest of creeping bent grass on golf course greens and tees all over the world, and sporadically fairways (Capinera, 2008). Furthermore, it is considered as a serious pest of vegetables and causes damage by feeding on basal stems of vegetable seedlings, underground roots and tubers (Rings, Arnold & Johnson, 1975). Other crops are known to be damaged by cutworms such as corn (Archer & Musick, 1977b; Johnson et al., 1984; Showers, Kaster & Mulder, 1983), wheat (Jacobson & McDonald, 1966; Jacobson & Peterson, 1965) and tobacco (Cheng, 1971). Adults do not cause damage and feed by sucking nectar from flowers. The young larvae (first and second instars) start feeding on leaf blades of turfgrass both day and night. When they develop to mature, older larvae (Third to sixth instars), they become nocturnal and develop a subterranean habit, producing silk-lined holes in the turf thatch or soil (Capinera, 2008).

1.2.5 Sciaridae (Sciarid flies)

This group of insects is generally small with a narrow body and long legs. The majority of them are dark in color with clear wings and distinctive venation. Until recent years, studies on Sciaridae were not popular among British Dipterists due to complications in their identification and the occurrence of most members of the family at one time (Menzel, Smith & Chandler, 2006). In Europe, sciarids have been identified as *Sciara militaris* through laboratory breeding of the larvae and then rearing them to adulthood. However, recent studies in Japan by Sutou, Kato and Ito (2011) used the DNA barcoding method to identify species and reported the larvae of Sciaridae (army worms or black fungus gnats) as *Sciara kitakamiensis*. The study also concluded that the identification of sciarid flies using on DNA barcodes is an effective method of identification in Japan. Menzel *et al*. (2006) reported that the sciarid fauna of the British Isles comprises 263 species including the following genera: *Bradysia, Bradysiopsis, Camptochaeta, Claustropyga, Corynoptera, Cratyna, Ctenosciara, Epidapus, Hyperlasion, Leptosciarella, Lycoriella, Phytosciara, Pnyxia, Pseudolycori-ella, Scatopsciara, Schwenckfeldina, Sciara, Sythropochroa, Trichodapus, Trichosia, Xylosciara,* and *Zygoneura.*

The larvae of sciaridae (fungus gnats) usually exist in animal dung but are possibly not primarily coprophagous (Curry, 1994). Clusters of larvae are found mainly in the (F) layer of the litter, sometimes in contact with the (H) layer and even the leaves but in most cases not the surface (Deleporte, 1981). The larvae of some species such as *Bradysia ocellaris* (Comstock) and *Lycoriella ingenua* (Dufour) are considered as major pests of mushrooms through damaging

compost, mycelium and sporophores. Moreover, adults of both species act as vectors for the introduction of mites and fungal diseases in cultivated mushrooms (Shamshad, 2010). Other studies by Braun, Sanderson & Wraight, (2012) suggested that larvae of *Bradysia impatiens* (Johannsen) have a potential ability of vectoring Pythium Root Rot pathogens. In South Devon grasslands, Benefer *et al*. (2010) found that the larvae of the families Sciaridae and Tipulidae comprised the second biggest dipteran families (20%) in grassland soils after Bibionidae (28%).

1.3 Population ecology of soil insects

The below ground insects can be characterized as “any insect which at one time or another, in the course of its development from the egg to the fully mature adult, spends one stage or stages of its life–history either on the surface of, or buried in the soil” (Cameron, 1913 cited in King, 1939). In a broad sense, the group involves almost all insects of grassland; ruderal and other communities where the plant life is relatively short (King, 1939). The abundance of insects in the soil has not been investigated widely and offers a particularly interesting field of research (McColloch, 1922). However, there are several studies that have investigated populations of soil insects. For example, in a census of animal life, McAtee (1907) surveyed an area of four square feet of soil to the depth that a bird could scratch and estimated that there were 3,012,079.2 animals, primarily insects per ha of woodland and meadowland. Additionally, the tangible remains of 1,210,210.4 dead insects per ha of woodland and 594,133.7 per ha of meadow land were found. In a study on the fauna of permanent pasture in Cheshire, Morris (1920) elucidated that “The census of insects actually found in the samples of soil gave

an insect population of 3,586,088 per acre (0.404 ha). The family best represented in the number of individuals was the Bibionidae, species of which made up 32–34 per cent, of the total number of soil insects. The next in number were the Mycetophilidae 16 –17 per cent., and the Staphylinidae 12–20 per cent. With regard to the number of species occurring in the soil, the Coleoptera, with 29 species, was the best represented order". Others have reported a mean population density of 522500, 669251 and 766000 leatherjackets/ha^{-1} for Northern Ireland, south-west Scotland and south-west England respectively, 65,000 to 865,000 leatherjackets/ha^{-1} from seven sites in Fermanagh, UK (Blackshaw, 1984), and 100,000 to 600,000/ha^{-1}in Northern Ireland (Blackshaw, 1983a; Blackshaw & Perry, 1994).

King (1939) Indicated that soil insects as a group are distinguished by slow mobility, low resistance to physical factors and inferior susceptibility to natural enemies specifically parasites and diseases. Subterranean insects, particularly those in environments that are undisturbed, are likely to be concentrated toward the surface. Even within an individual ecological community, the population of soil insects is far from being either regular or stable. Quantity, quality and distribution change extensively both in space and time. Active variation appears infrequently for some species but others show more frequent daily and seasonal changes. Lateral and vertical changes appear as a result of differences in either the appearance or growth of food or in other environmental factors, all related together as "soil heterogeneity" (King, 1939). The changes that regulate the abundance and distribution of a population trough time and space create a population system (Berryman & Kindlmann, 2008). Individual members of the

population, changes elucidating population size and structure, mechanisms that influence population size and structure and the environment are fundamental components of this system. These principles of the population system considerably regulate the capacity of the population to conserve itself within a changing landscape variation of habitable and inhabitable patches (Schowalter, 2011).

1.4 Population dynamics

Insect populations can vary dramatically in size through short periods of time, given their short generation times and large reproductive rates. Under unfavourable conditions, insect populations can decline for prolonged periods or even become extinct (Schowalter, 2011). The factors that influence insect population dynamics and the impact of insects on ecosystem conditions and services have been the focus of entomological research. Thus, characterizing population conversion are the most progressed methods for insects of economic importance (Schowalter, 2011). Price *et al*. (2011) has drawn attention to the fact that population dynamics have been a great field of interest in insect ecology for two main reasons:

Firstly, population dynamics includes variation in population through time and landscape. The subject acts as a principal topic in ecology in a combining approach that spreads throughout the science. Thus, it is critical to the theoretical advance of insect ecology;

Secondly, it is directly relevant to issues in managing plants as resources for humans, in agriculture, horticulture, and forestry.

In favourable environmental conditions, insect populations expand until limiting factors become depleted and eventually stop population growth (Schowalter, 2011). The population fluctuation varies according to population type, for stable populations, the fluctuation is relatively slow over time whereas irruptive and cyclic populations exhibit vast fluctuations (Schowalter, 2011). Miller & Spoolman (2011) stated that the population growth of some species can irrupt periodically to a high peak and then reduce to a more persistent lower level or, in several cases to an inferior, unfixed level. Various insects have irruptive population cycles that are related to seasonal fluctuations in weather or nutrient availability (Miller & Spoolman, 2011). In a study of the population changes of wireworms in six grass fields, Ross, Stapley & Cockbill (1947) have shown an increase in the number of wireworms sampled during winter months with twice as many recovered as those taken in the summer months. Cherry (2007) has expressed a similar view that the population of wireworms sampled monthly from June 2004 to June 2006 in sugar cane fields was significantly less in summer than those taken from other seasons and related this to environmental factors that influenced oviposition behaviour. Thus, the population dynamics of individual insects may vary depending on species and/or climatic variations. For example, in Diptera, a study on the populations of adult fungus gnats and shore flies in British Columbia by Keates, Sturrock & Sutherland (1989) showed that populations of both species were greater in summer than in winter in a nursery environment.

Other populations exhibit regular cyclic fluctuations, or boom and bust cycles through a time period whereas some others have irregular fluctuation in population size with no returning pattern. Some scientists characterize this

variability to disorder in such systems whereas others argue that it may be related to catastrophic population crashes resulting from sporadic severe weather in winter (Miller & Spoolman, 2011). Myers (1988) observes that there are several explanations for cyclic population dynamics, involving climatic cycles and differences in insect gene frequencies or behaviour, the quality of the food or disease susceptibility that arise during large changes in insect abundance. Schowalter (2011) reported that insect population cycles may be triggered by changes in climatic cycles which directly influences mortality or indirectly influences host condition or susceptibility to pathogens. Reference to Ashby (1979), suggests that deficiency in food resources over an outbreak may require a time lag for the undermined resources to restore to levels which are adequate in sustaining regenerated population increase. Royama (1992) indicated that interaction among controlling factors may play an important role in determining synchronized cycles. Liebhold, Koenig & Bjørnstad (2004) discovered that synchrony may emerge from three principal mechanisms which are:

A – Dispersal among populations decreases the size of large populations and increases nearby small ones.

B- Congruent dependence of diverse populations on an exogenous synchronizing random factor, such as temperature or precipitation or correlated environmental disturbances (so called “Moran” effect) (Ranta et al., 1997).

C- Trophic interactions with demes of other species, that is spatially synchronous or mobile.

Schowalter (2011) demonstrated changes in population size in four phases comprising endemic phase, release phase, peak phase and the declining phase, and also reported that demes of many species change at sizes that are inadequate to cause economic damage and thus do not draw attention. Several species may experience noticeable outbreaks with changes in environmental conditions, such as climate change, introduction to new habitats, or large-scale metamorphosis of habitual ecosystems to managed ecosystems (Schowalter, 2011).

1.5 Factors affecting population regulation

Populations which exhibit large fluctuations have an insubstantial natural ability to modulate growth. Population crashes may result from many factors that allow populations to build up beyond successive thresholds (Schowalter, 2011). These factors can modify the population size in two principal methods (Begon, Mortimer & Thompson, 1996; Price, 1997; Raffa *et al*., 2008).

a- If the number of organisms influenced by a factor is stable for any population number, or the influence of the factor does not depend on population number, the factor is considered as density –independent impact.

b- If the number of organisms influenced by density, or the impact of the factor, relies on population size, the factor is considered as a density dependent factor.

Schowalter (2011) observes that population size generally is transformed by abiotic factors such as climate and disturbances; however, it is sustained toward an equilibrium level by density–dependent biotic factors.

1.5.1 Abiotic factors

1.5.1.1 Impact of microclimate

Wallwork (1976) reported that the impact of microclimate on the soil fauna is mainly a direct one. There are also indirect impacts; for example, when the quality of vegetation is influenced by climate or via weathering development and the quality of the soil, these are considered as “Microclimatic effects” (Wallwork, 1976). Hodkinson & Bird (1998) and Bale *et al*. (2002) suggested that soil fauna may be buffered from changes in climate, and root herbivores are possibly less reactive to changes in climate than aboveground phytophages. In contrast, Pearce-Higgins, Yalden & Whittingham (2005) suggested that the development of invertebrates can be largely influenced by slight changes in temperature, so the phenology of root feeding species may be altered by even small rises in temperature. Wallwork (1976) states that microclimatic conditions may indirectly affect the dispersal of predators through acting on the distribution of prey. This could explain the way that an abiotic factor “microclimate” can interface with a biotic factor “food” to regulate the distribution of the soil fauna. Soil microclimate can merge with other biotic impacts such as intraspecific competition for habitat or breeding sites (Wallwork, 1976). Andersen (1987) and Brown & Gange (1990) indicated that root feeders can significantly be affected by any decline in soil moisture that affects feeding and oviposition behaviour, survival and abundance, notably during the growing season when peak activity appears for numerous herbivorous insects. Turning to Johnson & Murray (2008), one finds that root herbivory will be influenced directly through changes in climate, primarily through decline in soil moisture and temperature in the upper soil profile. Curry (1994)

confirmed that weather particularly temperature and moisture are of major importance influencing population density because of two main reasons:

1- It has a direct impact on metabolic process and growth rates of individuals and populations.
2- It has an indirect impact on the food supply and the general conditions of the habitat.

1.5.1.1.1 Temperature

Resh & Cardé (2009) observed that the distribution pattern of insects can be varied by changes in temperature which affect the dispersal of host plants and the insects that live on them. Coulson *et al*. (1976) used reciprocal soil core transfer between sites with different temperatures along an altitude gradient in the Pennines, North England and found that the decline in temperature with an average of 2 °C changed the duration of pupae from 23 to 42 days for *Molophilus ater* Meigen (Diptera:Tipulidae), and from 20 to 22 days for *Tipula subnodicornis* Zetterstedt. Pearce-Higgins, Yalden & Whittingham (2005) showed that the appearance of Tipulidae is noticeably associated with May temperature. A rise in temperature of 1 °C resulted in an earlier emergence peak of 7 days, and their emergence is predicted to be 12 days earlier by end of the 21st century. This could have an important implication in breeding birds that depend on them as a food source for breeding chicks (Pearce-Higgins, Yalden & Whittingham, 2005). Wilkinson & Daugherty (1970) Investigated the impact of constant and variable temperatures on the development of *Bradysia impatiens* (Diptera: Sciaridae), and found that the “Optimum constant temperature for the mean development of the

egg, larva, pupa, and longevity of the adult at constant temperatures was 29.4, 23.9, 29.4, 12.8°C, respectively. The optimum temperature required to complete development from egg to adult was the variable temperature 18.9-30.0°C. At this temperature regime, the mean developmental period was 19.2 days. At constant temperatures, the mean development period ranged from 48.8 days at 12.8°C to 19.9 days at 29.4°C. Fecundity was about the same from 12.8 to 29.4°C; however, fecundity was reduced at 32.2°C. The highest temperature tested was 32.2°C and had injurious effects on eclosion of eggs, development of larvae, emergence of pupae, and longevity of the adult."

Archer, Musick & Murray (1980) studied the impact of temperature and moisture on the development and reproduction of black cutworm (Lepidoptera: Noctuidae) and found that temperature was the only factor affecting the growth of all stages. The optimum temperature was 27°C and considerable increase in fecundity was recorded at 27°C and 20°C. The optimum treatment was 20°C for reproduction and survival whereas the majority of females were unsuccessful in laying fertile eggs at 34°C and 13°C (Archer, Musick & Murray, 1980).

Changes in temperature are also predicted to alter the seasonal movement of wireworms (Fisher, Keaster & Fairchild, 1975). Fisher, Keaster & Fairchild (1975) suggested that the seasonal vertical movement of wireworms within the genus *Melanotus* spp. Escholtz can be cyclic while the seasonal movement of *Condorus* spp. Escholtz can be noncyclic but is associated with soil temperature. The work of Campbell (1937) revealed that the activity of wireworms increased at high temperature and dormancy occurred at low temperature whereas extremes of either caused mortality. Lafrance (1968) found that temperature influences the

movement of wireworms. When the soil temperature reached 19.4°C, the larvae moved downwards and only a few larvae remained in the top layer of the soil. When the temperature reached above 22.2°C, this downward movement remained up to the end of August, and then the larvae moved up to feed close to the surface of the soil until mid-November when the cold started again (Lafrance, 1968). Campbell (1937) and Falconer (1945) indicated that the previous temperature experience of individuals influences the movement of wireworms (*Agriotes spp* and *Limonius californicus*). The movement activity of *Agriotes spp* was directly correlated with temperature in a range of 8-25 °C whereas the decline in temperature from 16 to 6 °C considerably increased the activity of wireworms (for 2 hr) in comparison with wireworms held at a constant 6 °C before the experiment (Campbell, 1937; Falconer, 1945). Falconer (1945) concluded that this response was in anticipation of further decreases in temperature to lethal limits.

1.5.1.1.2 Moisture

Apart from responses to seasonal variation in soil temperature, soil insects exhibit shorter term patterns of mobility toward and away from the surface of the soil in reacting to rainfall, irrigation, and soil drying accordingly (Villani & Wright, 1990). Peshin & Dhawan (2009) reported that phytophagous insects access water only from food material in their host plants. Consequently, they have developed several mechanisms to save water. Despite this, dry air may be lethal for many insects. Conversely, excessive moisture may act as a negative factor for insects via stimulating disease outbreaks, influencing natural growth, and reducing their capacity to tolerate low temperature (Peshin & Dhawan, 2009). Moreover,

moisture influences the reproduction rate but different insects have different abilities to tolerate conditions with high drought and a very wet environment (Peshin & Dhawan, 2009). Wallwork (1976) indicated that moisture can have a horizontal impact on the distribution pattern of soil fauna. For example, the family Carabidae commonly known as Ground Beetles are habitual in many types of grassland in north temperate regions. They are most common in moist sites. They also exist in wet marshlands and semi-arid steppe. The population density and the number of species decline in both at extreme rates of moisture. In more arid conditions, this family is displaced by other beetles belonging to the family Tenebrionidae (Wallwork, 1976). The occurrence of Carabidae varies according to genera; *Harpalus, Calathus* and *Cymindis*, are restricted to dry sites whereas *Agonum* species are restricted to wet or very moist sites. Other genera such as *Clivina*, *Carabus, Bembidion* and *Feronia* are categorized as Mesophilous and eurytopic insects. Many species of this group may also occur with xerophilous and hygrophilous species (Wallwork, 1976).

In a four year study of the relation of temperature and moisture on the seasonal movement of wireworms in the virgin organic soils of southern Quebec, Lafrance (1968) revealed that the majority of elaterid larvae shifted from the subsurface to the top 25.4 cm of the soil at the beginning of May when the temperature was 1.7 °C at a depth of 10.16 cm. The insects moved upwards and reached a peak when the top-soil moisture reached 200% and temperature at 10.16 cm depth was 12.8 °C (Lafrance, 1968). In a study to investigate the temperature and moisture preferences of wireworms, Campbell (1937) observed that wireworms of the species *Limonius* (*Phelates*) *californicus* Mann have rather clear habits toward

moisture and temperature. They avoided dry soil and when maintained in dry soil, they died shortly of desiccation. In contrast, saturated soil entirely stopped their activity and occasionally caused death (Campbell, 1937). Lees (1943) has expressed a similar view that moisture has a differential impact on the burrowing activity “ortho-kinesis” of wireworms within the genus *Agriotes* Esch. and they tend to migrate rapidly from dry sand and aggregate in wet sand (Lees, 1943). The variation in the resistance of soil insects to moisture loss may vary according to different life stages and morphological characteristics (Villani & Wright, 1990). Jones (1951) concluded that water shortage causes weight loss in the wireworms *L. cannus* and *L. californicus*. Additionally, adults and eggs were found to be the most resistant stages to desiccation followed by pupae and old larvae whereas pre-pupae and newly hatched larvae were the less resistant stages. The water content was decreased on transformation to the adult stage, and females contained less moisture than males (Jones, 1951).

Some Tipulidae species are also known to be susceptible to desiccation (Byers, 1961; Freeman, 1967; White, 1951). Milne, Laughlin & Coggins (1965) concluded that population crashes of *T. paludosa* Mg. larvae (third instar) and adults between August and September is due to high mortality from desiccation of eggs or first instar larvae. Meats (1967) found that the larvae (first, third and fourth instar) of *T. paludosa* grow highly at pF 0.8 but have zero growth at pF 4.2. In addition, the larvae require water from food to increase net weight and balance water loss from the cuticle when they are growing in turf with a pF above 3.77 (Meats 1967). Recent studies by Lepage *et al.,* (2012) suggest that edaphic factors particularly soil temperature and moisture affects soil dwelling insects and

the most susceptible stages commonly are eggs and early stages of larvae. Turning to Archer, Musick & Murray (1980), one finds that soil moisture does not affect the development of black cutworm (*Agrotis ipsilon* Hfn.) larvae (Lepidoptera:Noctuidae); however, the first five instars are influenced by relative humidity (R.H). Larvae needed from 6 to 9 instars to finalize development (Archer, Musick & Murray, 1980).

1.5.2 Biotic factors

1.5.2.1 Density dependent factors

The intra and interspecific interactions between species for limited resources and predations are known as the primary density-dependent biotic factors (Schowalter, 2011). Resource limitation has an important role for the survival of all organisms. Similarly, when competition on limited resources increases, fewer species get the chance to survive, reproduce or disperse (Schowalter, 2011). Predators, parasitoids and parasites play an important role in prey population dynamics. Predator populations increase with the increase of prey abundance until they get satiated and they respond both numerically and behaviourally to changes in prey population (Schowalter, 2011). Blackshaw & Petrovskii (2007) concluded that changes in larvae of *T. paludosa* Mg. populations are limited or regulated by density dependent negative factors at both local and regional scales. Furthermore, Blackshaw and Moore (2012) showed a marked decline in numbers of leatherjackets starting from April to September and linked them to biotic and abiotic factors for the first to third larval instars, but suggested the fourth larval instars were largely affected by density dependent biotic factors alone and the majority of mortality occurred at this stage. Unlike predators, parasitoids on the

other hand are not satiated when feeding on prey but their outbreak is limited by natural epizootics (Schowalter, 2011). The beetle family Carabidae including the genera *Brachinus*, *Lebia*, and *Lebistina* are the best known genera of parasitoid carabids feeding on variety of invertebrates for instance; larvae of *Lebia* species are ectoparasitoids on chrysomelid beetle pupae, flea beetles (Alticinae) and casebearers (Cryptocephalinae) suggesting a host-parasitoid relationship between them (Capinera, 2008).

1.5.2.2 Impact of Food

Curry (1994) concluded that food supply, in particular food quality has a significant role in limiting invertebrate populations and the area of plant that can be consumed by herbivorous insects is critically limited by the physical and chemical characteristics of plants. The majority of insects are capable of consuming a limited range of plant species and may be limited to specific parts of host plants that can support a suitable quality of food for a very limited period of time (Curry, 1994). Painter (1936) indicated that the seasonal appearance of insect species correlates with the seasonal presence of its food, and they can be synchronized by the availability of food (Painter, 1936). Wallwork (1976) reported that many of the soil dwelling insects have grasses and weeds as their natural food, and obviously have been able to respond significantly with additional food supplies such as cultivated crops. For example, in old grassland soils, wireworms appear in large populations.when these soils are ploughed over and planted with other crops (for example potatoes, onions, lettuce, celery, beet, mangolds, swedes, grass, oats, and wheat), the population of wireworms within the genus *Agriotes spp* increased continuously as pests of these crops (Wallwork 1976).

Zacharuk (1963) suggested that differences in sense organs in eleven species of wireworms belonging to the subfamilies Lepturoidini, Pyrophorini, and Elaterinae resulted in variation in their nutritional preferences (Zacharuk, 1963). The lack of host specificity is not only typical for wireworms, but many dipteran larvae exhibit the same character, as pointed out by Wallwork (1976). The larvae of Tipulidae particularly those related to *T. paludosa* and *Nephrotoma maculata* are well-known British pests particularly in grassland soils; however, they cause damage to other plants such as spring cereals, swedes, potatoes, turnips, strawberries, loganberries, blackberries, raspberries, maize, clover, brassica crops, peas, beans, lucerne, carrots, celery, lettuce, sugar beet, sweet corn, berries, tobacco, various vegetables and ornamentals (Blackshaw & Coll, 1999). Additionally, other insects such as cutworms in particular those related to the genus *Agrotis spp.* have a wide host range including beans, various root crops such as potatoes, beet, swedes and turnips, grasses and cereals in smaller proportions (Wallwork, 1976).

1.5.2.3 Natural Enemies

Curry (1994) demonstrated that natural grasslands play an important role in the normal organizing of invertebrate communities through providing a wide range of predatory and parasitic species. Nonetheless, information on this subject is limited. The majority of information on the impact of natural enemies belongs to the pest species in well simplified crop monocultures and is of questionable relevance for more heterogeneous natural systems (Curry, 1994). Apart from the impacts of climatic conditions which have been considered to be more important than natural enemies in regulating insect populations (Blackshaw, 1991), there

are several natural biocontrol agents that influence larval population of leatherjackets including vertebrates, viruses, bacteria, nematodes, and fungi (Table1.5) (Blackshaw, & Coll 1999). In a study to assess the potential of *Steinernema spp.* entomopathogenic nematodes (EPN) and the bacterium *Bacillus thuringiensis* subsp.*israelensis*, Oestergaard *et al*. (2006) indicated that both biocontrol agents have a potential role in decreasing leatherjacket populations particularly if they are applied during the early instars. Moreover, the success of *S. carpocapsae* was related to temperature > 12 ºC (Oestergaard *et al.*, 2006). A study by Campos-Herrera *et al*. (2006) found a new strain of *Steinernema feltiae* (Rhabditida: Steinernematidae) from the larvae of *Bibio hortulanus* (Diptera: Bibionidae) known as A2RLFP type through using PCR-RFLP profiles and sequence analysis of the ITS region of rDNA. Hominick *et al*. (1997) reported that entomopathogenic nematodes that belong to the families Heterorhabditidae and Steinernematidae are destructive parasites of insects with a global dispersion in the soil. Ram *et al*. (2008) claims that although EPN are lethal for many soil dwelling insect pests, they fail to be used in biological control programs because they are not sustained in sufficient numbers to provide persistent biocontrol. This is due to different factors including abiotic conditions, the quantity and quality of the host and movement rate which influences the chance of its persistence. Additionally, the study also suggested that further ecological studies on naturally occurring EPN may develop a full understanding of using EPN in the biological control and managing of soil dwelling insect pests (Ram *et al.*, 2008).

Carter (1974) found that the Tipula Iridescent Virus (TIV) is infectious to all larval stages, pupa and adults of both sexes of *T. oleracea* but the early stages particularly the first and second larval instars were more susceptible than the third and fourth larval instars. Carter (1978) further Investigated the role of TIV on the population decline of *Tipula spp* larvae in grassland as a biocontrol agent and the results suggested that the virus has the ability to infect tipulid larvae but with low efficiencies (Carter, 1978). Blackshaw & Coll (1999) reported that the larvae produce resistance to this virus if entered via the mouth, the common mode of transmission. Thus, it was considered that resistance to TIV is the reason for infrequently observing epizootics in the field (Blackshaw & Coll, 1999). Chapman (1994) investigated the role of the predatory ground beetle *Pterostichus melanarius* (Illeger, 1798) in decreasing the population of leatherjackets in laboratory conditions and found that *P. melanarius* has the ability to reduce the numbers of tipulid larvae. Turning to Kabaluk *et al.* (2005), they studied the role of the fungus *Metarhizium anisopliae* as a biological control agent for wireworms and found that the use of *M*. *anisopliae* causes mortality of wireworms which were lured toward the treatment area via wheat seed. Although some wireworms survived, they were seen to be infected when a great number died after incubation in the laboratory. The granulated conidia accommodated the greatest localized concentration of conidia, and thus caused the greatest mortality (Figure1.4) (Kabaluk *et al.*, 2005). Furthermore, some other parasites were recorded on *A. obscurus* such as the fungus *Beauveria bassiana* and *Tolypocladium cylindrosporum*, a parasitic fly, Mermithidae nematodes and deutonymphs of mites in the family Acaridae; but these mites were phoretic and

non–parasitic to the larvae (Kabaluk *et al.*, 2005). Peshin & Dhawan (2009) pointed out that the population of insects may increase rapidly when introduced into new geographic areas when they escape their natural enemies. The substantial conclusion being that natural enemies are regulating native populations (Peshin & Dhawan, 2009).

Table1.5 Natural enemies of Tipula species. Adapted from Blackshaw & Coll (1999).

Type of organism	Species / family	Development stage affected
Vertebrate	*Talpa europeae* L. *Strunus vulgaris* L. *Ciconia spp*. *Corvus spp*. *Vanellus vanellus* L. *Pyrrhocorax pyrrhocorax* L. *Chardadriiformes spp*.	Larvae
Insects	Tachinidae *Siphona geniculata* (de Geer) *Phaonia signata* (Meig.) *Pterostichus melanarius* (III.)	Larvae
	Anaphes sp.	Egg
Nematodes	Mermithidae , Thelastomatidae *Neoplectana carpocapsae* Weiser *Panagrolaimus tipulae* n.sp. *Rhabditis tipulae* n.sp. Steinernema feltiae Filipjev	Larvae
Fungi	Entomophthoraceae	Larvae + Adults T. paludosa
	Entomophthora arrhenoctona n.sp	Adults T. paludosa
	Entomophthora gigantean n.sp *Empusa caroliniana* (Thaxter)	Adults
Protozoa	Gregarinidae *Diplcystis tipulae* n.sp. Coccidia Microsporidia Rickettsiella tipulae n.sp. Entamoeba	Larvae
Virus	Tipula Iridescent Virus (TIV)	Larvae (T. oleracea +T. Paludosa)
	Tipula Nuclear Polyhedrosis Virus (NPV)	Larvae
Bacteria	*Bacillus thuringiensis* var. *Thuringiensis* (Berliner)	Larvae T. paludosa
	Bacillus thuringiensis var. *Israelensis* Goldberg and Margalit	Larvae

1.6 Spatial distribution of insects and distribution patterns

The spatial distribution of insect populations varies with the size of the population. Increased populations expand through a wider area when individuals in the high density core distribute to the edge of the population or settle new patches (Schowalter, 2011). Depending on the degree of habitat specialization, changes in the population of insects may be a valuable biological indicator of ecosystem conditions throughout landscapes or regions (Rykken, Capen & Mahabir, 1997). The spatial distribution of insects is generally classified as being random, contagious or regular (Figure1.5) (Chiang & Hodson, 1959; Kao, 1984; Ruesink & Kogan, 1994; Schowalter, 2011).

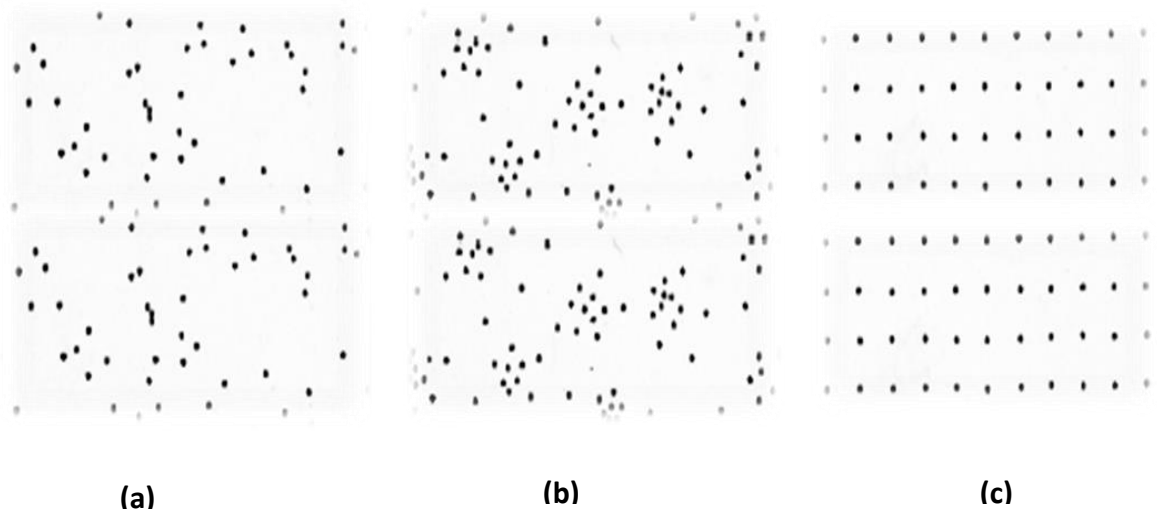


Figure 1.5 Different types of distribution a- Random, b- Aggregated and c- Uniform. Adapted from Kao, (1984).

1.6.1 Random spatial distribution

In a random distribution, the individuals show neither attraction to each other nor competition. In other words, the occurrence of one individual in a sample unit does not affect in any way the occurrence of other organisms in the same sampling unit. Sample densities exhibit a skewed 'Poisson' distribution. Many populations have a random distribution pattern (Kao, 1984; Schowalter, 2011). For example, wireworms within the genus *Limonius* spp (Onsager, 1969) and black cutworm (Story & Keaster, 1982a) in the soil.

1.6.2 Aggregated or clumped distribution

This results from congregation behaviour or choice for specific habitat area. This is characteristic of species that exist in herds, flocks, schools, etc. in order to boost resource exploitation or protection from predators (Schowalter, 2011). Mobile populations such as insects and animals aggregate as a result of for example; breeding, protection from enemies, supply of food or water (Kao, 1984). Schowalter (2011) reported that in this kind of distribution, the existence of an individual in a sample unit increases the probability of other individuals being found nearby. Populations with an aggregated distribution need extensive sampling in order to minimize the distinctly high variance in the number of individuals within sampling units and to provide a suitable representation with smaller sample sizes. This can be obtained by knowing the distribution of aggregation in different kinds of habitats (Schowalter, 2011). Aggregated distribution is common in many soil insects such as wireworms in the soil (Benefer *et al.*, 2010; Wadley, 1950), leatherjackets, Sciaridae, *Bibio johannis* and *D. febrilis* (Benefer *et al*., 2010).

1.6.3 Regular or uniform distribution

In a regular distribution, the occurrence of one individual in a sample unit decreases the probability of other individuals being nearby. The distribution will be more regular or uniform rather than being random (Metcalf & Luckmann, 1994). Kao (1984) reported that uniform or regular distribution may be an indication of competitive or antisocial behaviour. Schowalter (2011) indicated that the variability in mean density is low and the distribution tends to be normal. Therefore, the populations are easily monitored because even a small number of samples will represent the evaluation of mean and variance in population density in a similar way to those from a larger number of samples (Schowalter, 2011). Uniform distributions are infrequent in insects (Chiang & Hodson, 1959; Sevacherian & Stern, 1972). For example, corn earworm, *Heliothes* zea (Boddie) on sweet corn ear (Wolfenbarger & Darroch, 1965) and eggs of larch sawfly, *Pristiphora erichsonic* (Htg.) on shoots of tamarack (Ives & Prentice, 1958).

Schowalter (2011) reported that the distribution pattern changes depending on insect development, changes in population density or across spatial scales. For example, Salt & Hollick (1946) demonstrated that the spatial distribution of wireworms varies according to the larval stages; the small larvae exhibit a non-random distribution and are strongly aggregated whereas medium sized larvae are less aggregated and have a random distribution when they become large (Salt & Hollick, 1946). Benefer *et al*. (2010) demonstrated that the distribution pattern of phytophagous insect larvae in grasslands can vary depending on the scale of sampling through using three sampling scales (field, site and soil core scales). The distribution of wireworms *A. obscurus*, *A*. *sputator*, *A*. *lineatus* and

leatherjackets were aggregated at both field and site scales whereas more random at the core scale. In contrast, the distributions of Sciaridae, *B. johannis* and *D. febrilis* were aggregated at all scales, and random for non-Agriotes wireworms (Benefer *et al*., 2010).

1.7 Sampling Methods for subterranean insect Taxa in grasslands

In soil ecology, the population distribution of any taxa is correlated with the standard size of a soil sample. Nonetheless, knowledge on the importance of a sample unit size in understanding the patterns of spatial population of soil animals is little understood. The method used for sampling soil animals; in particular those related to the physical size of a sample and determining the position of a sample unit, may significantly influence the development of spatial surveys (Rossi & Nuutinen, 2004). Barberena-Arias, González and Cuevas (2012) and Benefer (2012) reported that differences in sampling methods for similar purpose affect the quality of the data and consequently the development of conclusions. various methods have been developed to collect soil insects from the soil such as using bait traps (Bynum & Archer, 1987; Parker, 1994; Parker, 1996; Simmons, Pedigo & Rice, 1998; Ward & Keaster, 1977) and different sized soil cores such as 10cm diameter with depth of 15cm (Benefer *et al*., 2010; Benefer, 2012; Blackshaw, 1994) or 6.5 cm diameter with depth of 5 cm (Blasdale, 1974). Scrimgeour (2008) has drawn attention to the fact that in soil science, soil sampling is essential to any field research program due to the impossibility of measuring total population in any practical study. For instance, a 10 ha field holds approximately 100,000 $1m^2$ soil pits or 1×10^7 of 10 cm^2 soil cores and thus total population sampling would be more of an irregular obsession than a scientific goal (Scrimgeour, 2008).

1.7.1 Sampling methods for leatherjackets (Tipulidae)

George (1966) reported that in the UK from 1959, estimations of leatherjacket populations showed that the larvae are of minor importance in many parts of the country. Since that time, a re-estimation of sampling strategy was suggested and a specialized group was established in those parts of the country in which leatherjackets were a substantial pest of agriculture including Northern, West Midlands, South-Eastern (Wye), South-Western (Bristol and Starcross) and Wales (Cardiff, Aberystwyth, Bangor). By 1961/1962 new methods of sampling were designed and continued with some modifications (George, 1966). In a survey to investigate the population of leatherjackets, Mayor & Davies (1976) used 6.3 cm core turf samples and a Bristol washing apparatus to recover leatherjackets. The study found that the population estimates of leatherjackets are equivalent to a 10.2 cm cores and there was a tangible relationship between percentage of rainfall in September and October, and mean annual leatherjacket populations in Gloucestershire, Somerset, Wiltshire and Dorset whereas in Devon and Cornwall, no such relations were observed (Mayor & Davies, 1976). Blackshaw & Petrovskii, (2007) concluded that the size of population changes monitored yearly in *T. paludosa* is due to limiting and regulating processes. Depending on field observations, it was concluded that these are not exclusive mechanisms (Blackshaw & Petrovskii 2007). George (1966) compared the efficacy of two sampling methods for estimating leatherjacket populations using an irritant emulsion of orthodichloro benzene (ODCB) to bring leatherjackets to the surface of the turf, and taking core samples then recovering leatherjackets in the laboratory via washing the cores. Although populations of leatherjackets were

higher when using soil cores, but no conclusions were made to support the efficacy of core samples over (ODCB) method. Milne, Laughlin & Coggins (1958) investigated the efficacy of the hot water process to recover leatherjackets from soil samples against the (ODCB) method and concluded that the efficacy of (ODCB) method is 85% when sampling from November to May. When the volume of the solution was doubled, the efficacy reached more than 90%. But this was dependent on observation of the sample for a minimum of 10 minutes and collecting leatherjackets that were brought to the surface. They also showed that some leatherjackets re-entered the turf and disappeared in over two minutes, thus indicating a further disadvantage of the method. George (1966) demonstrated that although (ODCB) gives a direct estimation of leatherjackets, however, the method is difficult to apply in arable fields when sampling, particularly during wet conditions or frosty soils. Alternatively, core sampling will be of significant importance if a large number of fields were to be sampled for experimental purposes (George 1966). Blasdale (1974) used a 6.5 cm diameter plastic pipe with a depth of 5 cm inserted in to the soil via a soil corer to collect leatherjackets from grass fields and developed a heat extraction method to expel the larvae from the cores. The heat was applied to the surface of the soil cores over 24 hours. The temperature gradient drove the larvae downwards into glass petri-dishes at the bottom of the apparatus which were partially filled with cold water (25mm). The larvae could then be collected and preserved for later identification. This method is known to be faster and less laborious in comparison with the ODCB method for extracting crane fly larvae from the soil and the sample size is 25 soil cores per field (Blasdale, 1974). Stewart & Kozicki (1987b) used a saturated brine

solution poured into 10 cm diameter plastic pipes that had been pushed into pasture turf. This caused leatherjackets to move upwards toward the surface of the soil via irritation. However, the method required a large volume of the solution to be carried across a field in the winter (Kozicki 1987). Blackshaw (1990) compared three sequential sampling plans with a standard twelve–pipe sample when sampling leatherjackets and concluded that a four pipe sequential sampling scheme decreases the average sampling time by 36% with no error increase.

1.7.2 Sampling methods for wireworms (Elateridae)

Several methods have been used to collect wireworms from the soil to estimate population levels. For instance, Finney (1946) used a set of sampling units including 10.16, 6.35, 4.48 cm soil cores and 15.24cm square units to survey wireworms in England and Wales. The study found that the distributions of wireworms within the genus *Agriotes spp* are not completely random and that small sampling units are more efficient than larger units. At low populations, all the sampling units were similar and wireworms had a Poisson distribution, but the differences became more important with large populations and a 6.35 cm soil core had advantages over both 10.16 cm cores and 15.24 cm square units but a 5.08 cm core had only a little advantage over 6.35 cm cores. However, the choice varies according to the soil quality and small cores may be difficult to use in heavy and stony soils (Finney, 1946). Smith *et al*. (1981) used different sized soil cores (22.9 cm, 10.2 cm, 7.6 cm, and 5.1 cm diameter) to estimate the population density of wireworms within corn fields in Missouri – U.S and found no significant differences between mean population densities of wireworms per m^2 for *Melanotus depressus* (Melsheimer), *M*. *verberans* (LeConte), and *Aeolus mellilus*

(Say) for the different sized cores. The study suggested that each of the core sizes investigated gives a similar estimation of wireworm populations (Smith *et al*., 1981).

The use of bait traps is also known to be an effective method of sampling for wireworms and detecting their presence in the field before planting (Lindroth, 2007). Toba & Turner (1983) studied the efficacy of different bait traps for sampling wireworms infesting wheat in Washington and showed that baits comprising a mixture of 1:1 wheat–corn, or wheat flour caught more wireworms than carrot, potato or wheat bran. Jansson & Lecrone (1989) evaluated the effect of bait traps to predict wireworm populations in potato fields before planting and suggested that oat meal-corn flake and rolled oat can be used successfully for pre-plant sampling of wireworms in potato fields in southern Florida. Simmons, Pedigo & Rice (1998) compared the efficacy of soil core sampling with six related methods for collecting wireworms including corn-wheat bait, melon bait, potato bait, wire-mesh bait, pheromone trap, and pitfall trap and showed that the corn/wheat bait is the most accurate method for estimating populations of wireworms in agricultural habitats and in revitalised conservation lands. Arakaki, Hokama & Yamamura (2009) studied the effect of bait traps to collect *Melanotus okinawensis* wireworms (Ohira) in sugar cane fields using different bait materials (sweet potato, potato, carrot and germinating rice seeds) and found that the germinating rice seed is the most attractive bait trap in comparison with other food material used in the study. Moreover, in semi-arid conditions, the attractiveness toward the baits increased by daily irrigation. However, sampling using bait traps tends to be less efficient during the presence of alternative food sources

particularly in freshly ploughed grass fields (Parker, 2001). Anon (1948) cited in Parker & Howard (2001) concluded that a 20 × 10 cm diameter soil core for an area between 4-10 ha is the most effective method of estimating wireworm populations.

1.7.3 Sampling methods for Bibionidae larvae

Skartveit (2002) reported that the larvae of Bibionidae live in groups and colonies in the upper layer of the soil and samples can be obtained by hand-picking the larvae from the soil layer or using traps such as Barber pitfall traps, Turnbull and Nicholls Quick traps, and by Tullgren extractions from soil samples (Skartveit, 2002). D' Arcy–Burt, (1987) stated that the larvae exist in the soil, thus they are subject to the difficulty of sampling correlated with various soil arthropods and in grassland no studies on sampling Bibionidae had been previously reported. Blackshaw & D'Arcy-Burt (1993) compared the use of different core sizes (5,10,15 , 21 cm diameter) for sampling Bibionidae with depths of 2,4,6,8,10 cm and showed that the smaller soil cores recovered more larvae per soil volume. The majority of larvae were found at 0-2 cm followed by 2-4cm with smaller numbers at 4-6 cm. No larvae were obtained at a depth of more than 6 cm. They concluded that a 10 cm diameter soil core with a depth of 6cm is an appropriate method for sampling Bibionid larvae (Blackshaw & D'Arcy-Burt, 1993). Recent studies by Benefer *et al*. (2010) reported high numbers of *B. johannis* larvae in grassland soils using a 5cm diameter soil core with a depth of 10cm and using a heat extraction method for recovering larvae from the soil.

1.7.4 Sampling methods for Sciaridae larvae

Deleporte (1981) used the quadrat sampling method to investigate the distribution of sciarid larvae in oak litter and found that the larvae of *P. falcifera* and *B. confinis* were distributed through the F layer of the litter and both species have an aggregated distribution pattern. Rutherford, Trotter & Webster (1985) studied the populations of sciard larvae in cucumber greenhouses using a 5 cm soil core sample and showed that the larvae appeared in the upper 5 cm of each 10 cm cucumber bag. Weekly samples showed a range of 0.0 to 1.1 larvae/ml and this was associated with populations of 2500 larvae per bag. Cabrera, Cloyd & Zaborski (2003) Studied the effect of monitoring technique to determine numbers of *Bradysia sp* larvae in growing medium using potato disks and carrot disks placed on the surface. The study suggested that potato disks are more efficient in determining Sciaridae larvae particularly third larval instars and increasing monitoring time from 24 to 48 h increased the numbers of recovered larvae. In arable lands, Nielsen & Nielsen (2004) studied the seasonal aspects of Sciaridae in two Danish barley fields, using emergence traps and recorded 480–527 individuals m^{-2} in cropped fields and 369–433 individuals m^{-2} in uncropped fields and suggested that the primary sex ratio of the species is “spanandrous” (in which males are only very sparsely found). Lee *et al*. (1999) used different sized sieves (30~140 mesh) to isolate larvae of *Lycoriella mali* Fitch infesting mushroom fields and found that larvae can be trapped with a 30~65 mesh sieve and the numbers were higher when using 42 and 65 mesh sieves. Zaborski & Cloyd (2004) used a method for extracting larvae of Sciaridae from soil-less growing media by fractioning samples using water floatation with an inverted flask

procedure then degassing the sediment under low air pressure and fractioning in magnesium sulphate (MgSO4) solution with a density of 1.12 g/cm^{-3}. The larvae were recovered from the surface of the $MgSO_4$ solution and preserved in alcohol. Recovery varied between larval instars and different types of media and was highest for fourth instar larvae (98-100%). Recently a 5 cm soil core sample with a depth of 10 cm has been used to collect larvae of soil insects in grasslands including Sciariadae and the heat extraction method to recover larvae from the soil (Benefer *et al.*, 2010).

1.7.5 Sampling methods for Noctuidae larvae

Whitcomb (1928) conducted an experiment for sampling cutworms in Waltham using Chickweed traps. 7707 larvae were collected which were dominated by larvae of *Euxoa ochrogaster* (Guenee), *Euxoa tessellata* (Harris), and *Euxoa perpolita* (Morrison) followed by *Agrotis jennica* (Tausch) and *Agrotis unicolor* (Walker) in smaller proportions (Whitcomb, 1928). In a survey of cutworms in Curlew Valley, Utah, Hanson & Knowlton (1973) used a one cubic metre soil sample in plots and found that the most abundant cutworms are the larvae of the genus *Euxoa spp* using a 1-10 cm mesh sieve for recovering larvae from the soil. However, this method was found to be time consuming and laborious for recovering larvae of cutworms from the soil (Hanson & Knowlton, 1973). Khinkin & Nikolov (1974) stated that for predicting the population and outbreaks of Noctuidae larvae (Cutworms), sampling needs to be taken in the autumn (September and October). The infestations are categorized as being low for 0-1 larvae m^{-2}, medium for of 1-3 larvae m^{-2} and high for 3 and more larvae m^{-2} (Khinkin & Nikolov, 1974). Archer & Musick (1977a) used apple pomace-bran

pelleted baits and pitfalls for sampling larvae of *A. ipsilon* in field corn. According to Danielson & Berry (1978) 929 cm^2 soil samples are adequate for estimating the population of *E. orchrogaster* in commercial-sized peppermint fields. The distribution pattern was contagious for most fields but became random when populations were smaller than 0.50/929 cm^{-2} (Danielson & Berry, 1978). Berry, Fisher & Shields (1981) reported that for most subterranean cutworms, a 929 cm^2 sample to a depth of 10.16 cm is sufficient for estimating their population density of the larvae. Story & Keaster (1982b) studied the use of bait traps (wheat seedling and wheat bran) for sampling larvae of black cutworm and found more larvae when compared with other sampling methods such as bait stations and bait burlap traps (Story & Keaster, 1982b). Lampert, Haynes & Cress (1982) evaluated the efficacy of four larval sampling methods for sampling white cutworms *Euxoa scandens* (Riley) in commercial asparagus fields using baited and unbaited pitfall traps, baited barrier plots and baited open plots. The study suggested that the open baited plot is the best method for detecting the larvae, whereas the baited barrier trap is the best method for estimating population density (Lampert, Haynes & Cress, 1982). Duffus, Busacca & Carlson (1983) evaluated different sampling methods for larvae of dingy cutworm, *Feltia jaculifera* (Guenée) using sack traps, pitfall traps and sieving. The study showed that sack trapping is the most effective method for sampling dingy cutworms as measured by relative net precision (RNP) whereas soil sieving needed more time to recover larvae and was less effective than sack traps (Duffus, Busacca & Carlson,1983). Story & Keaster (1983) found that reducing the size of bait traps from 1.98 to 0.41 liters does not affect the efficacy of the method for sampling black cutworms in

corn fields. Landolt *et al*., (2010) indicated that larvae of *Abagrotis orbis* (Grote) can be monitored through using a combination of pheromone traps including (Z)-7-Tetradecenyl acetate and (Z)-11-hexadecenyl acetate that can be extracted from the tip of female abdomen. However, no larvae were captured when using each trap separately. Moreover, several other species were captured with *Abagrotis* traps such as *Mamestra configurata* Walker, *Xestia c-nigrum* (L.), and *F.* which may complicate the use of this method for monitoring larvae of *A. orbis* (Landolt *et al*., 2010).

Macfadyen (1953) has drawn attention to the fact that despite the variation in the methods of extracting soil animals, they all have the same principle of the hand sorting method or two devices invented by Berlese in1895 and the floatation method elucidated by Balogh in 1948. None of the methods are known to be entirely effective in extracting soil animals, thus, since that time many variations have been developed in order to improve them but the fundamentals of these methods still remain with supporters and critics (Macfadyen, 1953). Finney (1946) pointed out that the inefficiency in extracting soil insects may arise from variation between field to field, day to day or from worker to worker, consequently influencing the unity of the results. It is the view of Fisher (1999) that for almost all invertebrates, no powerful methods have been developed by ecologists to assess geographic patterns of species richness, explicitly and areas of indigenousness.

1.8 Statistical approaches in spatial ecology

The use of statistics in analysing spatial data has become a challenge that is rather exclusive within the statistical sciences. Unlike many study areas, spatial statistics act as a gateway that brings together philosophies, methodologies and

techniques that are commonly taught separately in statistical curricula (Schabenberger & Gotway, 2005). During the 1950s, various methods of spatial statistics have been used with several modifications in order to determine and describe spatial patterns. These came from different fields of study including plant ecology, animal ecology, geography and mining engineering with similar or different goals such as explorative vs inference, mathematical approaches (variance-covariance vs count-based methods) and emphasizing assumptions (stationarity or pseudo-stationarity) (Fortin, Dale & Hoef, 2002; Liebhold & Gurevitch, 2002). According to Liebhold & Gurevitch (2002), in ecological data, deficiency in spatial independence is known to be an obstacle in understanding the biology of studied organisms. Recently, understanding, measuring and modelling of spatial patterns in relation to biotic responses have become critical viewpoints in the ecology of many organisms and systems (Liebhold & Gurevitch, 2002). Hoel (1943) reported that in biology, dispersion indices have a significant role in analysing the homogeneity of certain types of data in particular the binomial and Poisson distributions (Hoel, 1943). Myers (1978) studied the use of several distribution indices to investigate the distribution patterns of insect eggs and found that population density had no effect on Green's coefficient and standardized Morisita's coefficient whereas the variance/mean ratio has an insubstantial relationship with population density. Moreover, the study showed that the distribution indices belonging to K of the negative binomial are more or less aggregated than the negative binomial (Myers, 1978). Pena & Baranowski (1990) suggested that Taylor’s Power Law gives a better explanation of variance/mean ratio in comparison with Iwao's patchiness regression when they

investigated the spatial distribution of broad mite, *Polyphagotarsonemus latus* (Banks), and citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) on lime trees; both species were found to have an aggregated distribution pattern. However, the distribution pattern changed depending on the species (Pena & Baranowski, 1990).

In terms of mapping spatial data, Kriging methods (a set of geostatistical analyses) have been used for mapping spatial structure and patterns of spatial distribution of data (Beelen *et al*., 2009; Berke, 2004; Bostan, Heuvelink & Akyurek, 2012; Carrat & Valleron, 1992; Cressie & Kang, 2010; Gotway *et al*., 1996; Hengl, Heuvelink & Stein, 2004; Kleinschmidt *et al.*, 2000). Zhang *et al*. (2003) studied the use of the ordinary kriging method in ecological entomology using simulated models and showed that the two step spherical model is the best simulating model and the one step spherical model is more appropriate than the linear model (Zhang *et al*., 2003). Many other methods have been used for analysing spatial data such as nearest neighbour distance for measuring spatial relationships in populations (Byers, 1984; Campbell, 1992; Clark & Evans, 1954), spectral analysis for spatial patterning (Legendre & Fortin, 1989; Platt & Denman, 1975; Ripley, 1978) and fractal analysis for analysing the scales at which different organisms are interfacing with the patch form of a landscape (With, 1994). Fortin, Dale & Hoef (2002) reported that the use of spatial statistics varies according to the objectives of the research or the sampling design and measurement types of the data (Tables 1.6 and 1.7).

Moreover, spatial statistics also vary according to the kind of spatial structure with measuring or estimating methods (Dale & Hoef, 2002) including:

1- Global spatial structures such as variance/mean ratios and aggregation indices.

2- Spatial intensity and range as a function of spatial lags such as Ripley's k function, blocked quadrat variance methods, join count, Moran's I, Geary's c, Mantel test, Semi variance and SADIE.

3- Spatial interpolation such as Trend surface analysis, Kriging, splines, Voronoi polygons.

Table 1.6 Types of spatial statistics according to the objective of the study

Source: Fortin & Dale (2002).

Objective of the study	Spatial statistics
Exploration	Nearest neighbor, k-nearest neighbor Ripley's K (uni-and bivariate), Join count, Moran's I, Geary's c, semivariance y, mantel test (Multivariate)
Inference	Ripley's K (uni-and bivariate), Joint count, Moran's I , Geary's c , semi variance y, Mental test (Multivariate)
Mapping (Interpolation)	Trend surface analysis, kriging, splines, voronoi polygons.

Table 1.7 Types of spatial statistics according to the type of data measurement and sampling design (Fortin & Dale, 2002).

Sampling design	Categorical/qualitative	Numerical/quantitative
Exhaustive census (x –y coordinates)	Nearest neighbors k-Nearest neighbors Ripley's K (uni- and bivariate) Join-count	Aggregation indices (e.g. Variance/mean, etc.)
Regular spacing (1D and 2D)	Block variance quadrat Spectral analysis Wavelet analysis Fractal dimension	Moran's I, Geary's c, Getis (global and local) Semivariance SADIE Mantel test (multivariate) Trend surface analysis, kriging, splines
Irregular spacing (1D and 2D)	Fractal dimension	Moran's I, Geary's c, Getis (global and local) Semivariance SADIE Mantel test (multivariate) Trend surface analysis, kriging, splines, Voronoi polygons

In grasslands, different spatial statistical methods have been used to investigate the spatial distribution of soil fauna. Decaëns and Rossi (2001) used different spatial statistical methods including Partial Triadic Analysis (PTA), Principal Component Analysis (PCA), Kriging, Moran's auto correlogrames and Mantel's test to elucidate the spatial structure of earthworm communities and soil heterogeneity in a tropical pasture. They demonstrated that the earthworm community is stable in time, and is dependent upon the study period at temporal scales. Other studies by Rossi (2003b) and Decaëns, Jiménez and Rossi (2009) used a Partial Triadic Analysis (PTA) and Geostatistics when investigating the spatio-temporal pattern of grassland earthworms. The PTA calculated a

multivariate analysis of a set of data (variables) and separated the spatial pattern of earthworm populations that was typical for all the dates. Additionally, the results showed that earthworm populations are well-regulated and that the spatial patterns are stable through time (Decaëns, Jiménez and Rossi, 2009).

Perry (1995) introduced the Spatial Analysis by Distance IndicEs (SADIE) method to quantify spatial pattern of mapped and count data. The technique requires two dimensional coordinates for each sample unit for both mapped and count data (Perry, 1995). Rossi (2003a) used this method (SADIE) to study the spatial distribution of two earthworm groups in a grass savannah using 100 sampling points in a square grid and found that both groups are highly aggregated. Jiménez and Rossi (2006) used the same technique to investigate the spatial association or dissociation between two earthworm groups in a native savanna and a grass-legume pasture in the Colombian "Llanos". The study found that both species are well structured in grass savanna but were randomly distributed in the pasture field at nearly all sampling dates (Jiménez and Rossi, 2006). In a recent study, Richard *et al*. (2012) used the SADIE method to describe the spatial distribution of pasture earthworms in north western France and suggested that at local scales, the earthworms' spatial distribution are influenced by interspecific interactions. The SADIE has also been used to analyse the spatial distribution of soil dwelling termites and is considered to be more successful than multivariate analysis in analysing the association between termite spatial distributions and biotic and abiotic variables (Donovan *et al*., 2007). Holland *et al*. (2005) used this technique to study the spatial distributions of four Carabidae species in arable crops at a farm-scale and showed that all the species have an aggregated

distribution but with different patch sizes and locations among species. At the landscape level, Blackshaw and Vernon (2006) used SADIE to describe the spatial distribution of adult male *A. lineatus* and *A. obscurus* in an agricultural landscape and found that both species have an aggregated distribution pattern and they respond similarly to the landscape environment.

Zuur, Ieno and Smith (2007) pointed out that in case of many zeros and double zeros in ecological data, R and Q techniques can be applied as a measure of association to multivariate analysis of species data using the most appropriate statistical methods such as principal coordinate analysis (PCoA), non-metric multidimensional scaling (NMDS), the Mantel test or ANOSIM. Benefer *et al.* (2010) used NMDS to study species interactions of phytophagous grassland insect larvae at three different scales (core, site and field) and deviance partitioning to study the effect of environmental variables on soil insects. In addition, it was concluded that multi scale analysis can provide a better explanation of species distributions (Benefer *et al.,* 2010). Despite this, studies on the spatial distribution of subterranean insect taxa in grasslands using larger scales such as the farm scale are lacking. This information on the spatial dynamics of farmland invertebrates has a potential role in understanding the basic biology of important soil invertebrate pests, improved management of pests and biodiversity conservation in sustainable agriculture (Benefer *et al.*, 2010; Holland *et al*., 2005). Therefore, to do this, a relevant method of sampling is crucial in order to estimate population levels and determine patterns of populations between different insect taxa at different sampling scales. Moreover, the spatial scale of sampling as shown by Benefer *et al*. (2010), Levin (1992) and Wiens

(1989) has an important impact on observed species distributions and has been somewhat neglected in ecological studies (Benefer *et al*., 2010). Recently, in the UK a new national facility has been established by Rothamsted Research –The Farm Platform. This has created three hydrologically separated farmlets which undergo under different managing methods. As part of this process, the current study was undertaken in order to:

1- Investigate the spatial distribution of subterranean insect taxa in grassland using three different spatial scales (core, field, and farm-scale).
2- Find out if there is any relationship between insect distributions to each other and environmental variables in grassland soils.
3- Develop a molecular method to differentiate between the larvae of *Tipula paludosa* and *oleracea* using universal DNA primers.

Chapter 2

The spatial distribution of subterranean grassland insects

Chapter two: The spatial distribution of subterranean grassland insects

2.1 Introduction

Permanent grasslands support a habitat for many invertebrate species, most are bacterial, fungal and detritus feeding. Grassland Insects as part of this community vary in their feeding pattern and behaviour; therefore, the combined feeding of a pest complex leads to considerable damage and needs a sustainable pest management strategy (Klein *et al.*, 2007). Even within short time scales, the abundance of many insects may vary due to their small size and active reproductive rates and this has been linked to various environmental factors which could facilitate a demographic evaluation of insect populations and their response to environmental factors (Schowalter, 2011). Therefore, regarding soil insects as complex pests, there are many factors that play an important role in the distribution and diversity of soil insects in grasslands most importantly the temperature, moisture and food in addition to soil texture and insect response (King, 1939).

There have been various studies on the effect of these factors (see Chapter 1) on the abundance and distribution of soil insects. However, despite its importance, little is known about the effect of spatial scales on the distribution and diversity of soil fauna (e.g. Benefer, et al., 2010) as it is known to be a complicating factor affecting species dispersion (Sandel & Smith, 2009). Thus, understanding the spatial distribution and abundance of species at different spatial scales and the factors driving them is a vital subject in the spatial ecology of many organisms (Gambi et al., 2013; Ingels & Vanreusel, 2013).

The key indicator of understanding the patterns of species distributions relies in descriptions of the mechanisms which determine observed patterns. Commonly, these mechanisms act at a variety of scales rather than those on which patterns are observed. Thus, the study of changes in distribution patterns and variability under different scales is required alongside the development of measures for simplification, aggregation and scaling (Levin, 1992). Spatial structuring represents a powerful role in ecosystems and modelling spatial patterns using multiple and temporal scales is demanding (Borcard & Legendre, 2002; Borcard et al., 2004). The development of new methods of statistical analysis has also led to the development of methods of analysing spatial data to determine, for example, the influence of space on species richness and/or presence (Borcard, Legendre & Drapeau, 1992; Borda-de-Água et al., 2011; Cocu et al., 2005; Dormann et al., 2007). Moreover, the use of other methods such as indices of dispersion and multivariate techniques, spatial location methods (e.g. geographical coordinates) of samples to determine spatial patterns for instance, and deviance partitioning may help interpret species dispersal in agricultural systems (Benefer et al., 2010).

One of the most important pests of grasslands in the UK, is the larvae of Tipulidae commonly known as leatherjackets which feed on the roots of grasses and have caused a significant yield loss (see Chapter 1) in Northern Ireland with populations as low as 132,500 ha^{-1} (Blackshaw, 1984) and as high as 1.14 million ha^{-1} in south west Scotland (Johnson & Murray, 2008). Few studies in the UK have investigated the factors that influence the distribution of subterranean insects in grasslands such as wireworms (Parker & Seeney, 1997) and

leatherjackets (McCracken, Foster & Kelly, 1995), and, little is understood about the impact of scale and biotic factors on the species distribution (Benefer *et al.*, 2010). The impact of scale on the distribution of subterranean insects in agricultural grassland, species interactions and their impact on the distribution of subterranean insect larvae is scarce (Benefer et al., 2010). In addition, studies on the effect of scale on species distributions at the farm level are lacking. This information is crucial especially if insect pests associated with grasslands are to be managed; the spatial relationship between insects and their host plants remains a challenge (Ferguson et al., 2003). This has a potential role in pest and biodiversity management programs and in producing an appropriate predictive model (Benefer *et al.*, 2010). In terms of pest management techniques, this is particularly important for subterranean insects of economic importance due to the current developments in using pesticide alternatives on one side and restricting the use of synthetic chemical pesticides on the other side (Barsics, Haubruge & Verheggen, 2013)**.**

In the current study, soil insects of the family Tipulidae, Sciaridae, Chironomidae, Psychodidae, Dolichopodidae, Bibionidae, Chrysomelidae, Stratiomyidae, Carabidae (adults and larvae), Muscidae, Cantharidae, Elateridae and Noctuidae were sampled from grassland soils at Rothamsted Research North Wyke. The Tipulidae larvae (leatherjackets) and Elateridae (wireworms) are both common insects of grassland soils feeding on grass roots but when they appear in large abundance in arable lands, some species can cause visible damage to crop roots thus, they have been mainly investigated as agricultural pests (Benefer et al., 2010). Although the study of the effect of sampling scale on the distribution of soil

insects at core, site and field scales has been investigated by Benefer et al., (2010), studies on the farm-scale distribution of these taxa are lacking. In this study the spatial distribution of soil insects in agricultural grassland are investigated at three different sampling scales using a systematic sampling program.

The objectives of this study were to:

1- To investigate and characterize the spatial distribution of subterranean grassland insects on the Farm Platform at Rothamsted, North Wyke.

2- To study and calculate the relative population sizes and comparative distributions for each taxa.

3- To determine the effect of sampling scale by deviance partitioning.

4- To quantify any Inter-specific co-occurrence or antagonism between taxa.

2.2 Materials and methods

2.2.1 The study site and soil sampling

The study was conducted at Rothamsted Research, North Wyke near Okehampton (Geographic Location: Lat: 50.73237; Long: -3.99635) which is located in the heart of Devon`s farmland, on the northern edge of Dartmoor, UK. Soil samples were taken from 19 permanent grassland fields (>5 years age) including (Pecketsford, Longlands North, Longlands South, Longlands East, Great Field, Ware Park, Poor Field, Top Burrows, Burrows, Bottom Burrows, Orchard Dean, Golden Rove, Dairy North, Dairy East, Dairy South, Lower Wheaty, Lower Wyke Moor, Middle Wyke Moor and Higher Wyke Moor). The Fields ranged between 1.3 ha and 7.86 ha (Figure 2.1) (Total study area = 67.2 ha). Soils were clayey, brownish colours, mottled below 40 cm (Harrod & Hogan, 1981). Sampling points were taken from a 25 m sampling grid using ESRI ArcMap (V.10) GIS software for plotting points. Sampling points were determined using a GPS system (a Trimble R8 base station with a Trimble R6 rover + Trimble TSC3 controller). Samples were taken on the 15th of November 2011 and between April and May 2012 and repeated between 8th April 2013 and 8th May 2013. Soil cores were taken using a standard soil corer with a 6.5 cm plastic pipe which was inserted to the depth of 10cm. A total of 2260 soil samples was collected (1130 in each year) and put in plastic bags with labels and taken to the laboratory for processing and recovering of soil insects.

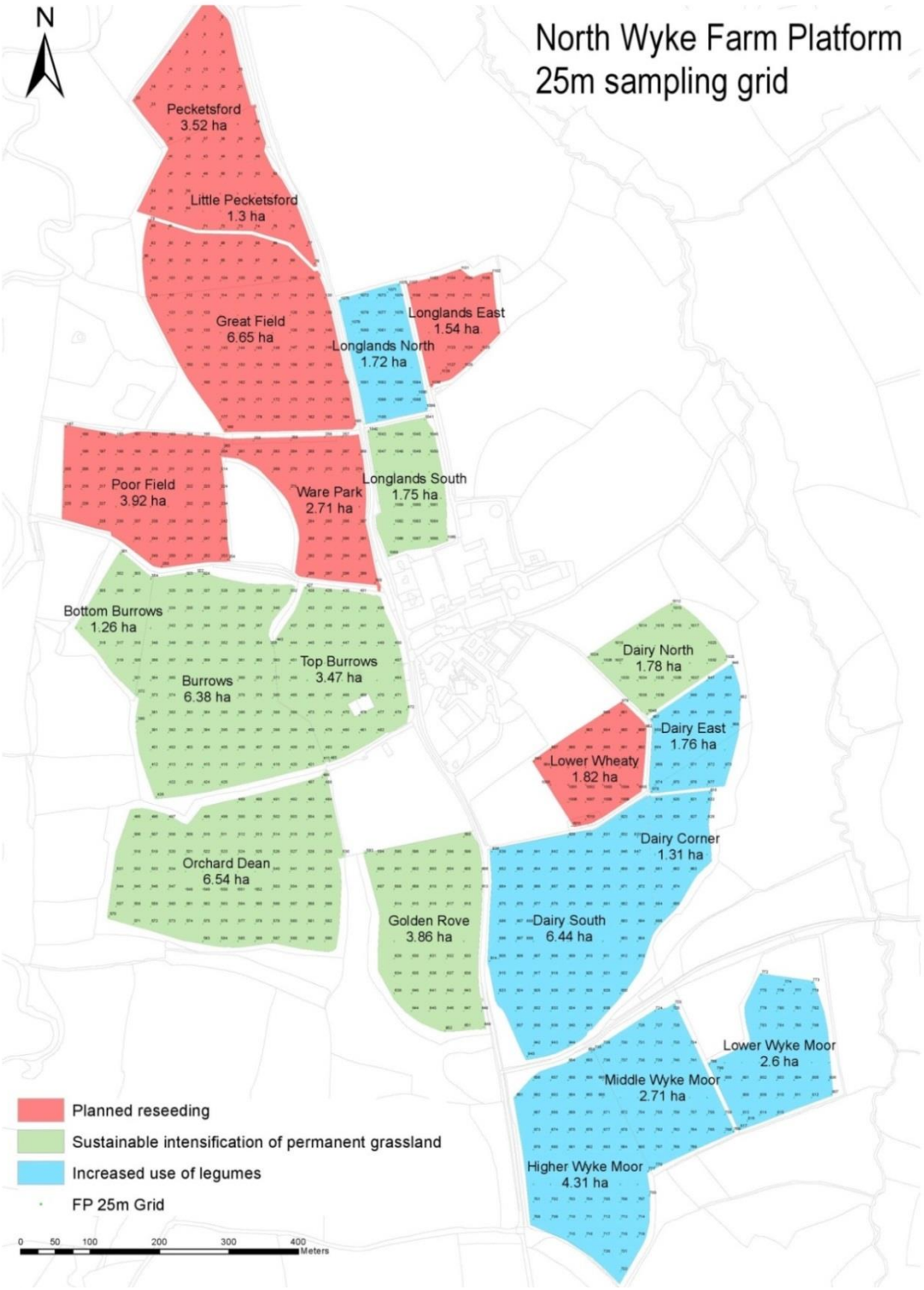


Figure 2.1 A 25m sampling grid of the total study area (67.2 ha) – North Wyke farm platform Rothamasted-Devon. Dots (small numbers) represent sampling points.

2.2.2 The extraction and Identification of soil insects

A modified heat extraction method based on Blasdale (1974) was used to recover soil insects from the cores. The apparatus consists of two layers, each layer holding 25 soil samples with some modifications such as putting sawdust between the plates from the surface and sides of the apparatus. The upper layer contained 25 holes fitted to the cutting cylinders (6.5 cm diameter) and below it a similar baffle plate with smaller holes in order to catch the cylinders and prevent them from sliding down to the dishes that contain water at the bottom of the apparatus. The cylinders were slipped gently through the holes with the turf surface downwards (Blasdale, 1974). The drawer was then slid into the heater with a temperature of 35-40°C at the surface of the turf. At 35 °C , the larvae particularly leatherjackets move away from temperature and start going downwards through the core over a period of 20-25min (Blasdale, 1974). Samples were left over 24 hours. A total of 512 insects including larvae and several adults were recovered (233 in 2012 and 279 in 2013) and put in 70% alcohol for later identification. Several subterranean insect taxa were recovered and then identified to family level using available morphological keys (Freeman, 1983; Luff, Turner & London, 2007; Stubbs & Drake, 2001). The families included; Tipulidae, Sciaridae, Stratiomyidae, Muscidae, Carabidae, Chironomidae, Psychodidae, Bibionidae, Chrysomelidae, Cantharidae, Elateridae and Noctuidae. There were also some other taxa which were unable to be identified at Plymouth University and therefore sent to the British Natural History Museum for identification including the families Scatopsidae, Anthomyiidae and two types of Dolichopodidae larvae:

Dolichopodidae (Type A): 4 posterior lobes, dorsal pair shorter than ventral (Campsicnemus).

Dolichopodidae (Type B): 5 posterior lobes with apical hair tufts, 4 equal sized with pair of elongate dark sclerotized patches, dorsal lobe smaller with one sclerotized patch (undescribed larva). In addition, there were some other unknown beetle larvae which were grouped together as unknown Coleoptera.

2.3 Data analysis

The spatial distribution of wildlife is often investigated through dividing the study area into regular sampling grids and collecting data on species abundance or presence/absence from some parts or all the squares of the grid (Augustin, Mugglestone & Buckland, 1996). Generalised linear models (GLM) can then be applied to model the spatial distribution of data by relating species abundance or presence/absence data to referenced covariates (Osborne & Tigar, 1992; Walker, 1990). The sampling points were determined through X and Y geographical coordinates (Easting and Northing) and a spatial distribution map were made for each individual taxa using SURFER (Golden Software - version 8). The distribution pattern of each taxon was determined individually at each scale (Farm, field and core) using variance to mean ratios (VMR) as an index of dispersion. This is suggested to be an approximation of the spatial distribution and primary patterns in the data depending on the number of specimens repeatedly dispersed for each sample unit (Benefer et al, 2010). Nonetheless, it is not considered as an indicator of the spatial pattern which does not use count data, but it uses the

spatial location such as geographical coordinates of the samples (Binns, Nyrop & Werf, 2000).

Analysis was done in R (version 3.0.1) using the Brodgar interface v.2.5.7 (Highland Statistics Ltd., 2006). Non-metric Multidimensional Scaling (NMDS) was applied to understand the association between taxa at each scale using presence/absence data. NMDS calculates the distance matrix and shows it in a low dimensional configuration (mostly two or three). The association between taxa at each scale (core, field and farm) was visualized using Jaccard's coefficient as a measure of similarity. The ordination was chosen to form the best number of dimensions or axes (m), with the lowest STRESS value as a measure of deviation from monotonocity (Kruskal, 1964). Plots were made between the Number of axes (m) and STRESS values of m. The optimal value of m coincides with a clear change in STRESS value (the so-called elbow effect) (Zuur et al., 2007). The smaller the stress value (<0.05), the better the configuration is whilst a STRESS value greater than 0.3 indicates a poor representation and more axes (m) will be required (Zuur et al., 2007). There were a high number of cores with no insects (zero counts) in both years' data, thus it was not possible to use geostatistical methods for the determination of spatial pattern because of this violation of the assumptions of these tests (Benefer et al., 2010). Alternatively, partial linear regression was used using a Generalized Linear Model (GLM) with a binomial distribution for species presence/absence data with the Logistic Link Function applied to partition the deviance explained by space, biotic influences (presence/absence data for all other taxa) and scale for each taxon (Benefer *et al.*, 2010; Legendre & Legendre, 1998; Lumaret & Jay-Robert, 2002). In order to

determine the proportion of deviance explained by sampling scale, scale as a nominal variable was divided further into Farm and field. In total, eight components of deviance were calculated: effect of biotic, effect of space, the effect of scale, combined effect of biotic and scale, combined effect of biotic and space, combined effect of scale and space, the combined effect of all three categories of variables and unexplained deviance.

2.4 Results

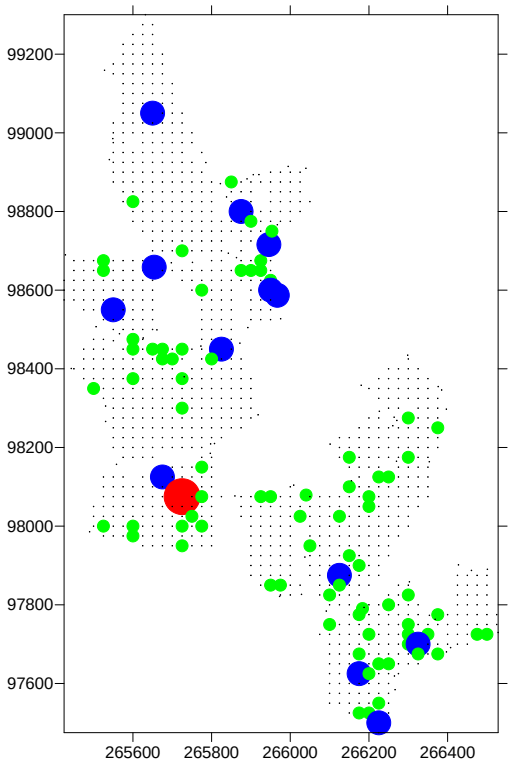
A-First year's data-2012

2.4.1 The abundance of taxa and distribution maps

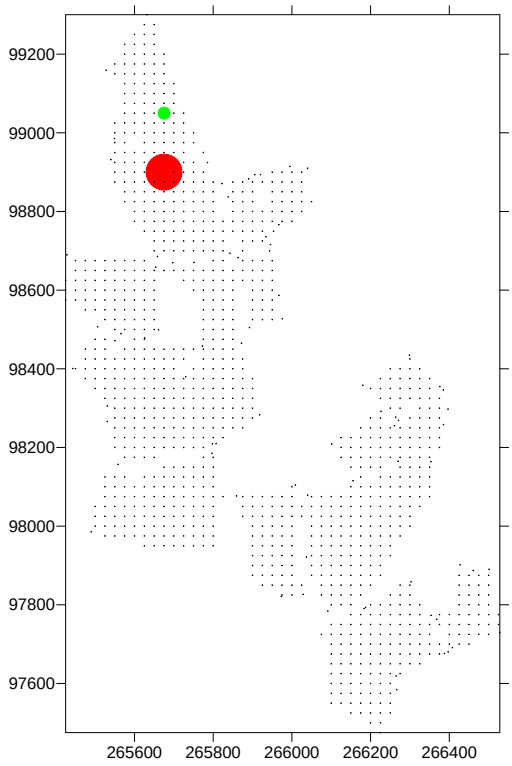
The larvae of Tipulidae were the most abundant dipteran species recovered, followed by Sciaridae larvae. The larvae of Stratiomyidae and Scatopsidae were the third largest group of dipteran species identified and were found in similar proportions. Carabidae (Adult and larvae) with Dolichopodidae (Type B) were also found in similar proportions followed by similar numbers of Muscidae and Dolichopodidae (Type A) and Unknown Coleoptera respectively. The families Chironomidae and Anthomyiidae were also found in similar numbers and composed the lowest numbers of all taxa (Table 2.1). The distribution maps are shown for each individual taxon (Figure 2.2 a-l).

Figure 2.2 The distribution maps of each taxa at the Farmpaltform. The insect count scales are Green=1, Blue =2 and Red = 3+ per core. The x and y numbers represent geographical coordinates of sampling points (easting and northing).

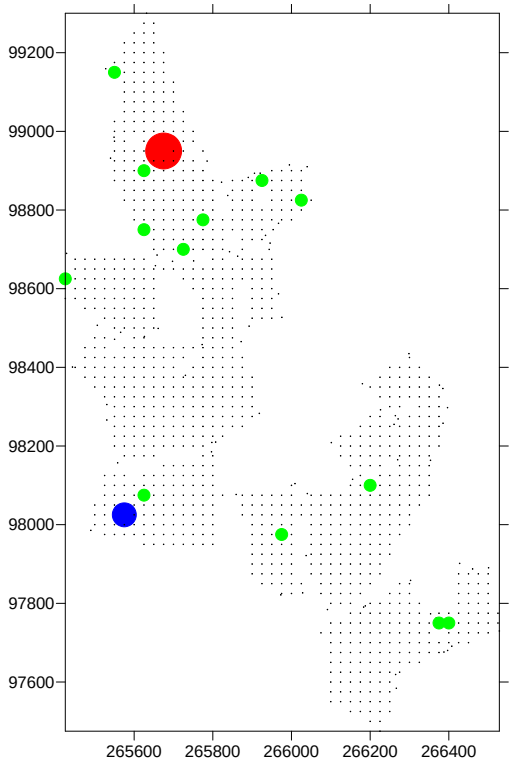
(a) Tipulidae



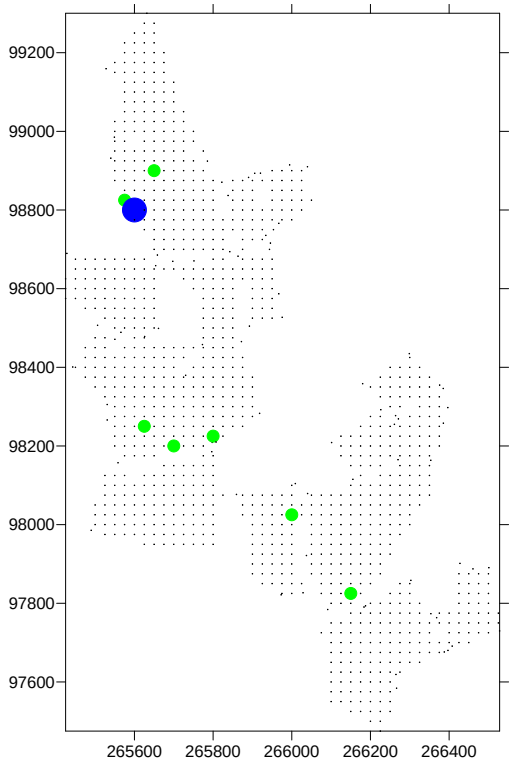
(b) Sciaridae



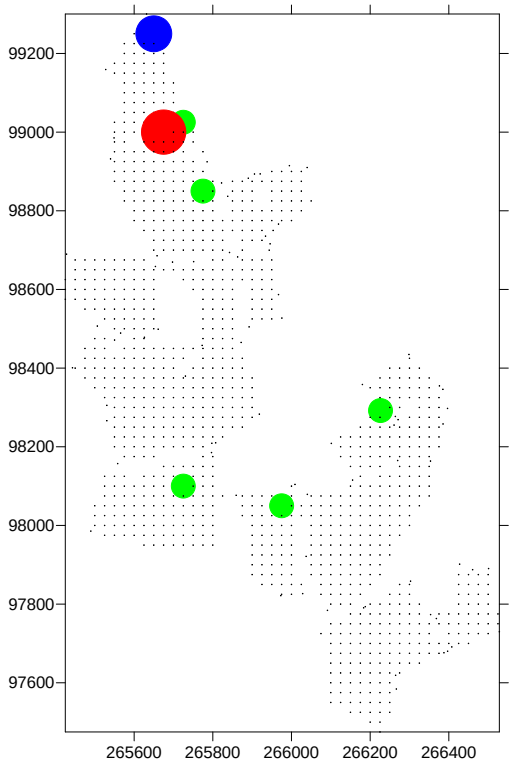
(c) Stratiomyidae



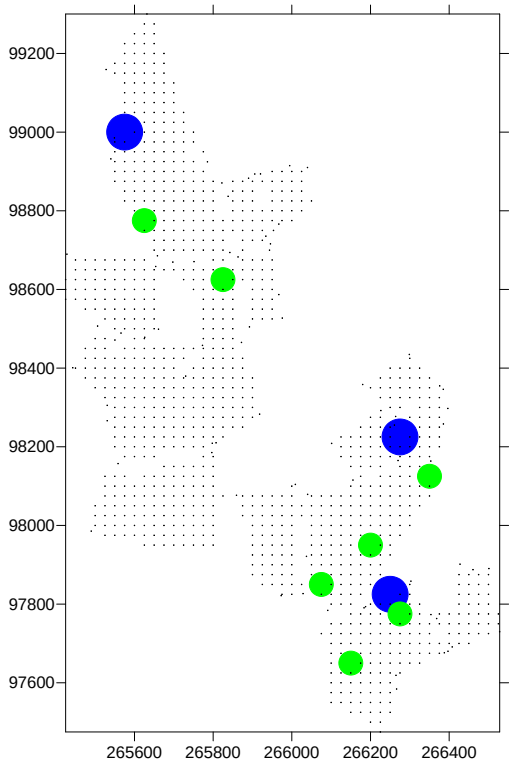
(d) Muscidae



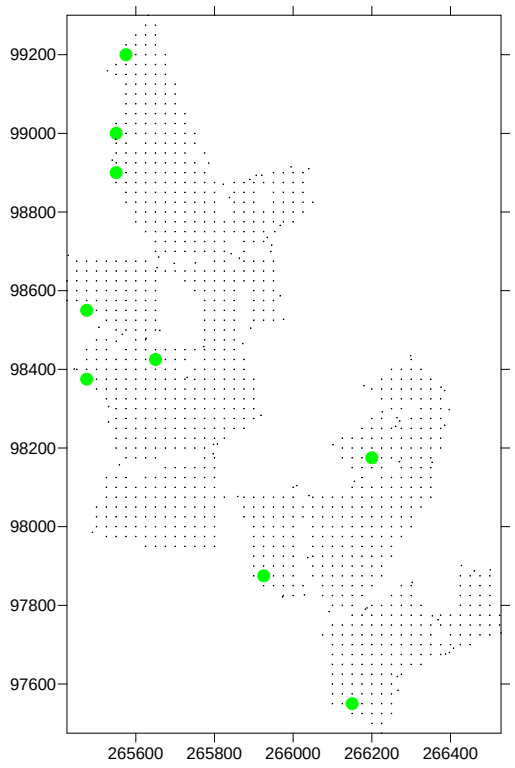
(e) Adult Carabidae



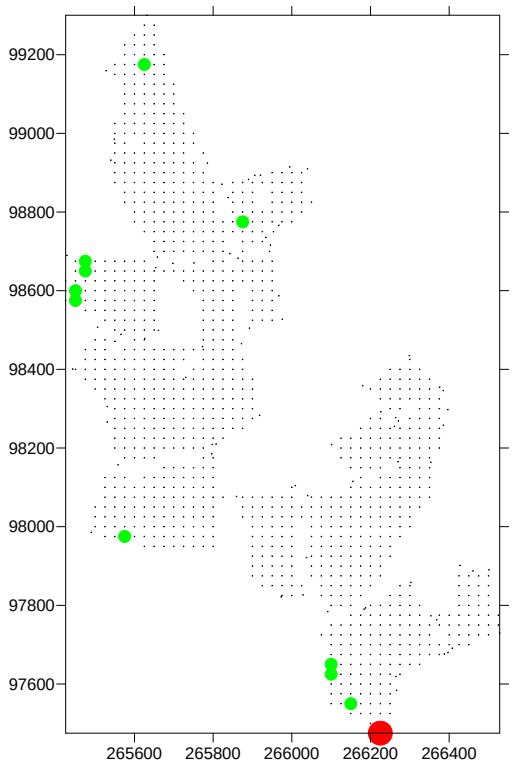
(f) Carabuidae larvae



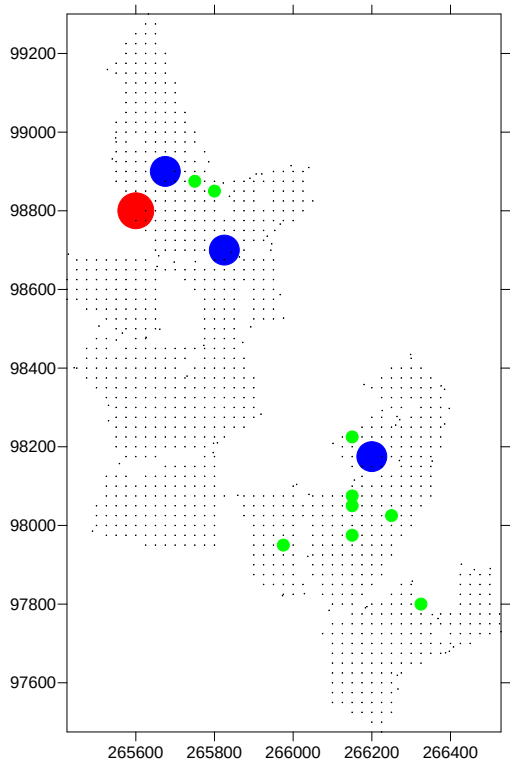
(g) Dolichopodidae (Type A)



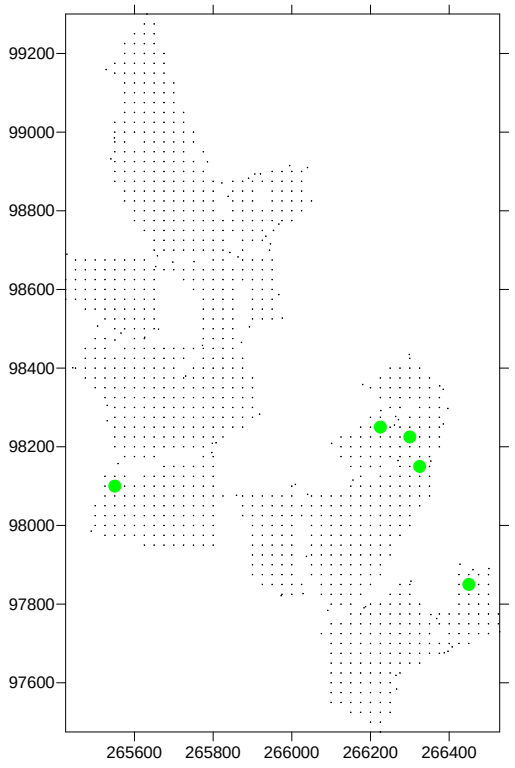
(h) Dolichopodidae (Type B)



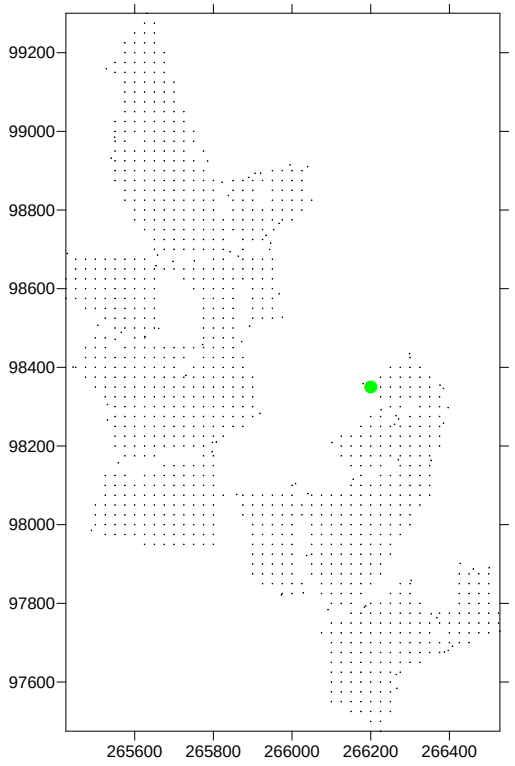
(i) Scatopsidae



(j) Unknown Coleoptera



(k) Chironomidae



(l) Anthomyiidae

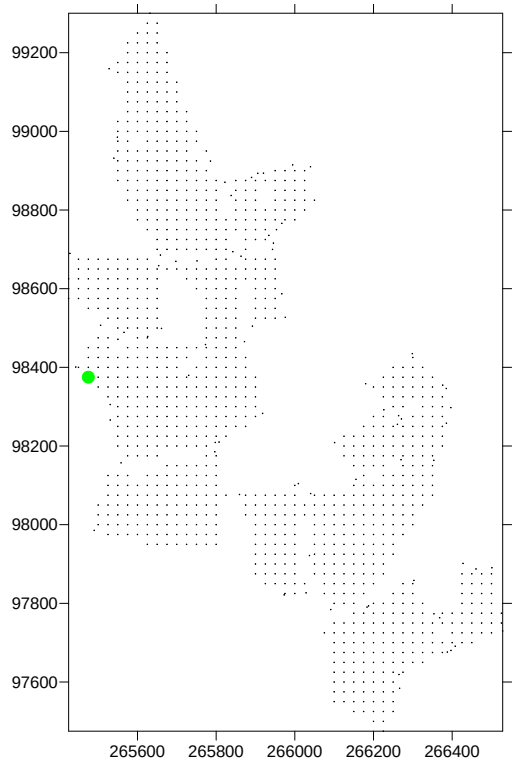


Table 2.1 Number of individuals of different taxa, their percentage of the total 233 insects obtained from 1130 cores over 19 fields in 3 farmlets (a group of six fields), and the population density based on an area of 8.88m^2 (Total area of soil cores taken from the study).

Taxa	No. of individuals	%	Population density (m^{-2})
Tipulidae	106	45.5	11.94
Sciaridae	27	11.6	3.04
Stratiomyidae	18	7.7	2.03
Muscidae	9	3.9	1.01
Carabidae (Adults)	13	5.6	1.46
Carabidae (Larvae)	13	5.6	1.46
Dolichopodidae (Type A)	9	3.9	1.01
Dolichopodidae (Type B)	13	5.6	1.46
Scatopsidae	18	7.7	2.03
Unknown Coleptera	5	2.1	0.56
Chironomidae	1	0.4	0.11
Anthomyiidae	1	0.4	0.11

2.4.2 Variance/mean ratio

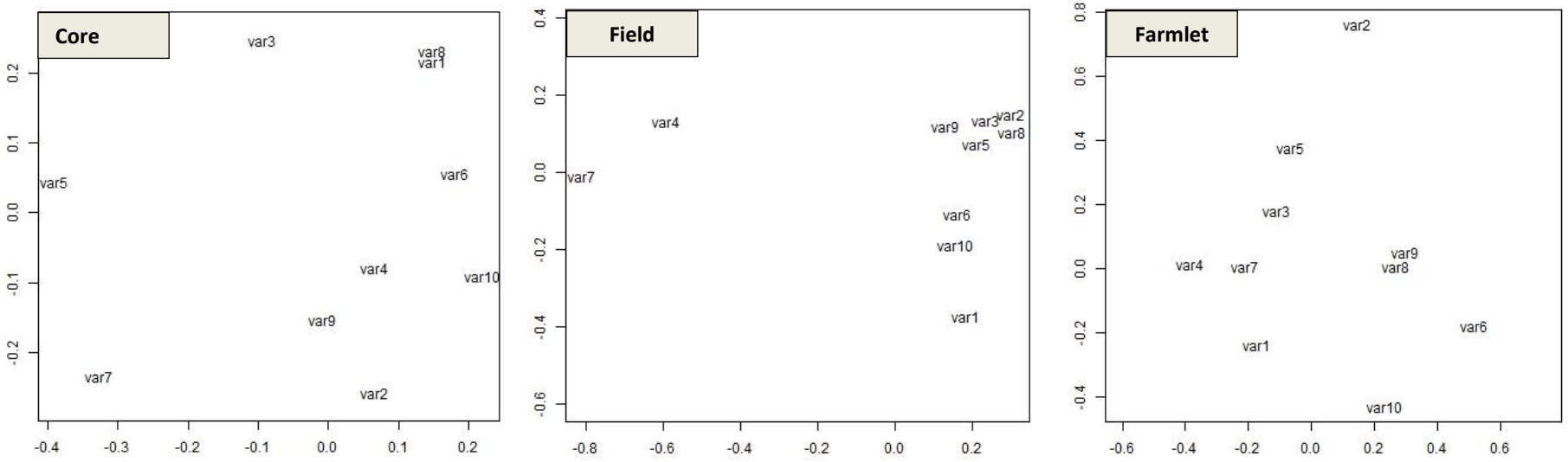
The variance/mean ratio (VMR) generally varied for the taxa at each scale (Table 2.2). Tipulidae, Stratiomyidae, Muscidae, Carabidae larvae, Dolichopodidae (Type B) and Scatopsidae were aggregated at the farmlet and field scale (VMR>1) but were more or less randomly distributed at the core scale (VMR=1). The family Sciaridae and adult Carabidae were aggregated at all three scales whereas the Dolichopodidae (Type A), Chironomidae and Anthomyiidae were randomly distributed at all three scales. The unknown Coleoptera were randomly distributed in the core and field scale but had an aggregated distribution at the farmlet scale.

Table 2.2 The variance/mean ratio for the taxa at the farmlets, field, and core scale.

Taxa	Farmlet	Field	Core
Tipulidae	13.2	4.5	0.6
Sciaridae	20.3	23.7	12.5
Stratiomyidae	3.6	2.8	0.7
Muscidae	1.4	2.1	0.6
Carabidae (Adults)	4.80	5.9	1.7
Carabidae (Larvae)	4.2	1.6	0.7
Dolichopodidae (Type A)	1.0	0.8	0.5
Dolichopodidae (Type B)	2.4	3.3	0.7
Scatopsidae	3.6	5.1	0.8
Unknown Coleoptera	2.2	0.8	0.5
Chironomidae	0.8	1.0	0.5
Anthomyiidae	0.8	1.0	0.5

2.4.3 Non- metric multidimensional scaling

NMDS ordination biplots (Figure 2.3) showed that the dispersal of taxa with reference to each other changed between the three sampling scales (Farmlet, field and core). Species close to each other are considered to co-exist regarding the geographical composition of the biplot (Zuur *et al.*, 2007). The distribution of individual taxa varied between sampling scales using NMDS. The family Tipulidae and Dolichopodidae (Type B) were found in close proximity at the core scale but they were dissociated at the field and farmlet scale (Figure 2.3). The families Sciaridae, Stratiomyidae, adult Carbidae, Dolichopodidae (Type B) and Scatopsidae separately and Carabidae larvae and unknown Coleoptera were found in close proximity at the field scale but they were dissociated at the core and farmlet scale except Scatopsidae and Dolichopodidae (Type B) which were also associated at the farmlet scale (Figure 2.3 field and farmlet). The distribution of other taxa varied across the three sampling scales and most were dissociated at each scale (Figure 2.3).



Dimensions= 2, Stress= 0.1298099 Dimensions= 2, Stress= 0.1002877 Dimensions= 2, Stress= 0

Figure 2.3 NMDS ordination biplot (axes 1 and 2) for core, field and farmlet scale. The close distances between taxa indicate coexisting species. The insect families are: var 1=Tipulidae, var 2=Sciaridae, var 3=Stratiomyidae var 4=Muscidae, var 5=, Carabidae (Adults), var 6=Carabidae (Larvae), var 7=Dolichopodidae (Type A), var 8=Dloichopodidae (Type B), var 9=Scatopsidae, var 10=Unknown Coleoptera.

2.4.4 Deviance partitioning between different sampling scales

The percentage of deviance explained through deviance partitioning in the dependent variables (presence/absence) varied between the three groups of variables (location, space and biotic) which ranged from 10 % for Tipulidae to 56% for Sciariadae (Table 2.3). Among the demonstrated variables, Scale was found to be the most important factor for all taxa explaining between 11% and 41% of deviance, whereas Space and Biotic separately had lower influences on most taxa. However, the farmlet scale individually comprised the least explained deviance for almost all taxa which were between 1 to 16% (Table 2.3). The effect of combined biotic and scale variables was relatively greater than the scale and space variables and the joint effect of biotic and space was small for almost all taxa in comparison with biotic and scale and scale and space respectively. The explained deviance between taxa revealed that the distribution of species was more influenced by field than farm scale. For example, field comprised the most explained deviance for almost all taxa including Tipulidae, Stratiomyidae, Muscidae, Carabidae (adults and larvae), Dolichopodidae (Type B), Scatopsidae and unknown Coleoptera. In contrast, the biotic factor composed the most explained deviance for the families Sciaridae and Dolichopodidae (Type A). For the core scale, it was excluded here due to the high number of zero counts (cores containing no taxa). Among 1130 cores, 172 cores contained only two different taxa (Diptera and Coleoptera) and no significant differences were found between observed and predicted co-occurrence of the species using a chi-square test (χ^2 = 3.39, df= 39, $p>0.05$). This reinforces that taxa at this scale are distributed randomly with reference to each other and population density.

Table 2.3 The percentage of explained deviance explained through deviance partitioning in the dependent variables (species presence/absence) between three groups of explanatory variables (location, space and biotic), their combinations, and the deviance unexplained by the variables in this study for each taxa.

Variables / Taxa	Biotic	Scale (all)	Scale (Farm)	Scale (Field)	Space	Biotic and Scale	Biotic and space	Scale and space	All variables	Unexplained
Tipulidae	2	11	3	8	3	10	4	8	10	90
Sciaridae	37	41	16	25	19	55	54	25	56	44
Stratiomyidae	1	19	3	16	2	17	3	18	19	81
Muscidae	13	19	2	17	3	26	15	21	29	71
Carabidae (Adults)	3	23	2	21	6	23	8	30	31	69
Carabidae (Larvae)	3	25	9	16	3	19	5	16	19	81
Dolichopodidae (Type A)	22	16	1	15	4	32	24	23	41	59
Dolichopodidae (TypeB)	5	30	4	26	5	30	9	35	38	62
Scatopsidae	17	26	4	22	2	35	21	22	37	63
Unknown Coleoptera	2	31	10	21	7	23	10	30	32	68

B- Second year's data-2013

2.4.5 The abundance of taxa and distribution maps

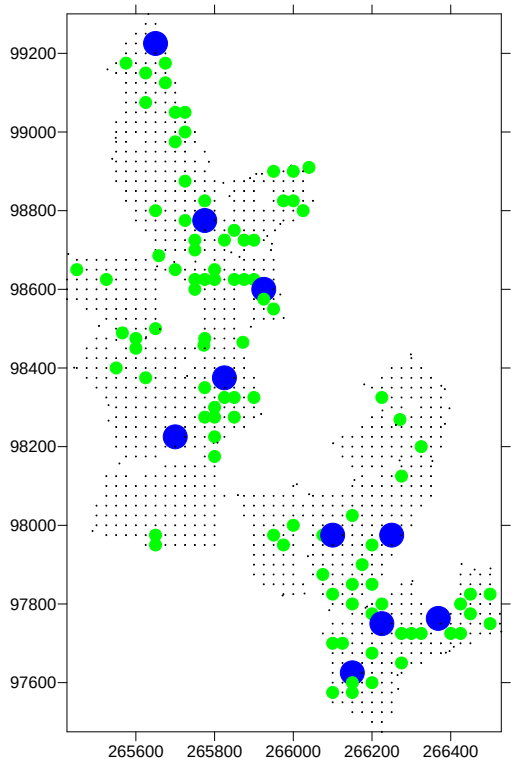
A total of 279 insects were recovered belonging to the families Tipulidae, Chironomidae, Psychodidae, Dolichopodidae (Type B), Bibionidae, Chrysomelidae, Stratiomyidae, Carabidae (Adults and larvae), Cantharidae, Muscidae, Elateridae and Noctuidae. The family Tipulidae were the most abundant species identified in the second year survey (2013) and comprised 41.9% of all specimens recovered followed by the Chironomidae, Psychodidae, Dolichopodidae (Type B) and Bibionidae respectively (Table 2.4). The families Chrysomelidae and Stratiomyidae were found in similar numbers with relatively small populations followed by Muscidae. Other families including Carabidae (Adults and larvae), Cantharidae, Elateridae and Noctuidae comprised the smallest number of the taxa recovered (Table 2.4). The distribution maps are shown for individual taxa in figure 4.1 a-m.

Table 2.4 Number of individuals of different taxa found in 2013 at the farm platform, their percentage of the total 279 insects obtained from 1130 cores over 19 fields in 3 farmlets, and the population density based on an area of 8.88m^2 (Total area of soil cores taken from the study).

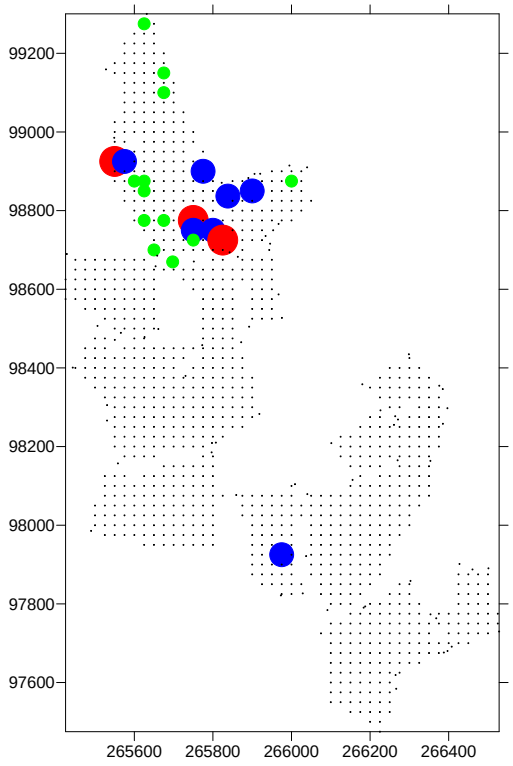
Taxa	No. of individuals	%	Population density/m^2
Tipulidae	117	41.9	13.18
Chironomidae	38	13.6	4.28
Psychodidae	29	10.4	3.27
Dolichopodidae (Type B)	27	9.7	3.04
Bibionidae	17	6.1	1.91
Chrysomelidae	13	4.7	1.46
Stratiomyidae	13	4.7	1.46
Carabidae (Adults)	4	1.4	0.45
Carabidae (Larvae)	6	2.2	0.68
Cantharidae	2	0.7	0.23
Elateridae	1	0.4	0.11
Noctuidae	1	0.4	0.11
Muscidae	11	3.9	1.24

Figure 2.4 The distribution map of each taxa at the Farm platform in 2013. The insect count scales are Green=1, Blue =2 and Red = 3+ per core. The x and y numbers represent geographical coordinates of sampling points (easting and northing).

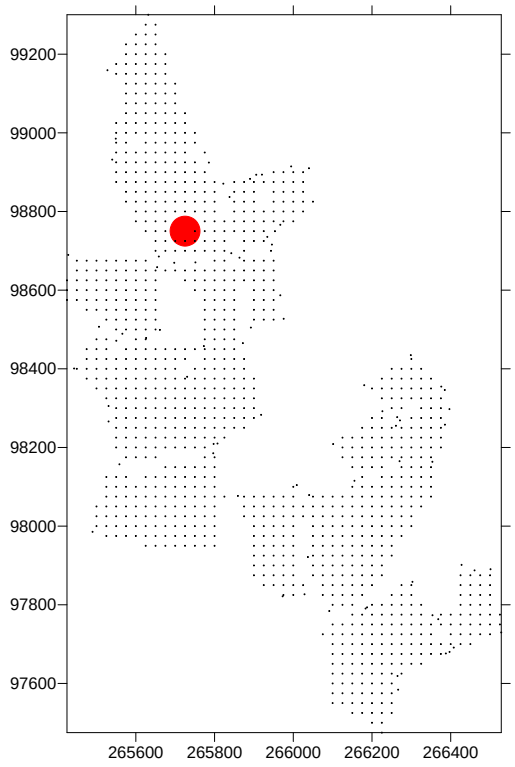
(a) Tipulidae



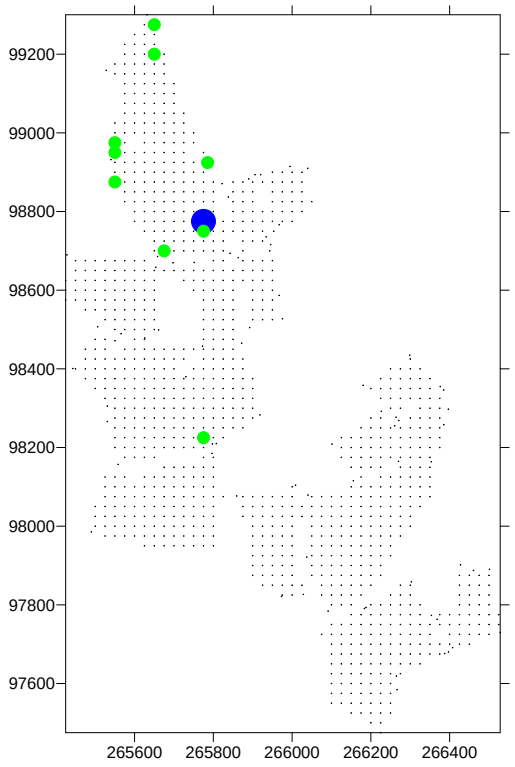
(b) Chironomidae



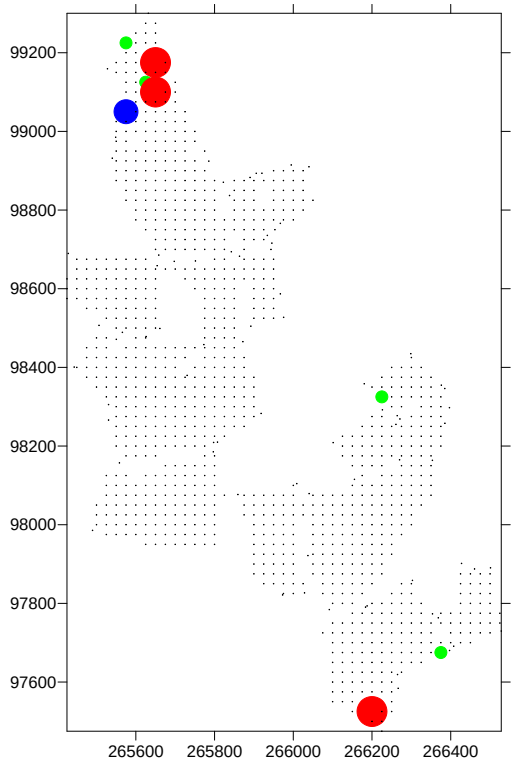
(c) Psychodidae



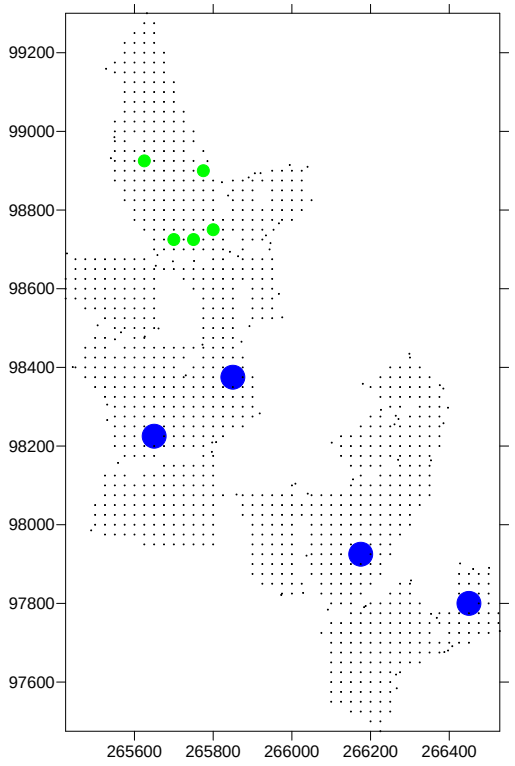
(d) Muscidae



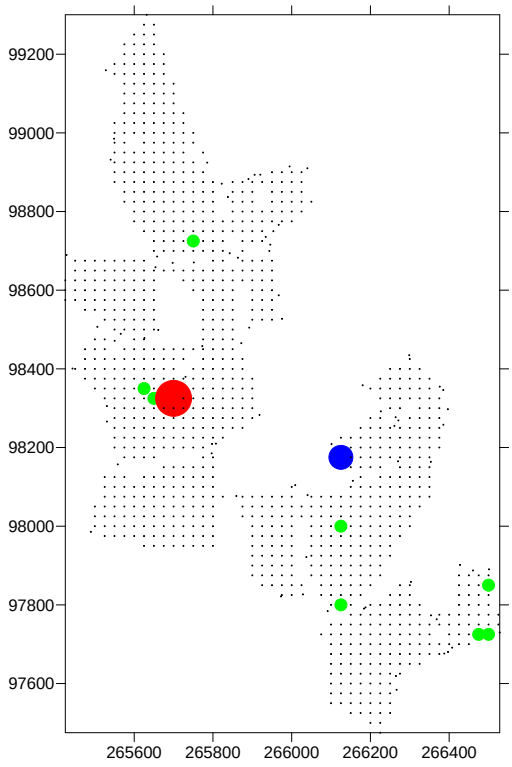
(e) Bibionidae



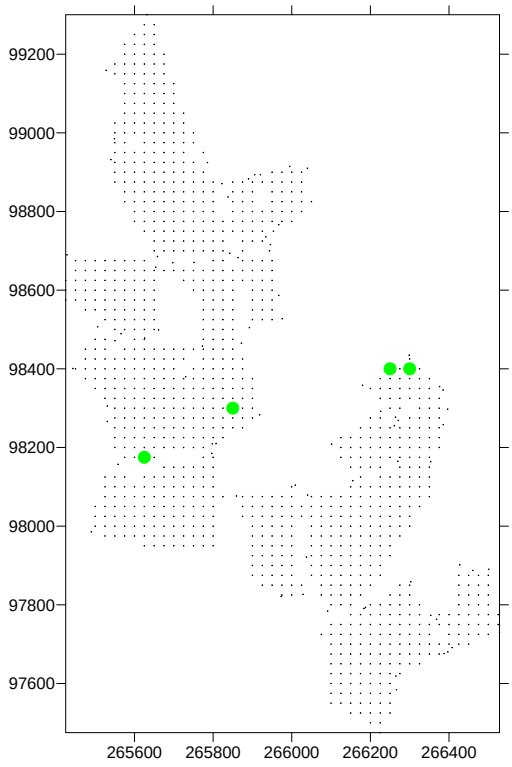
(f) Chrysomelidae



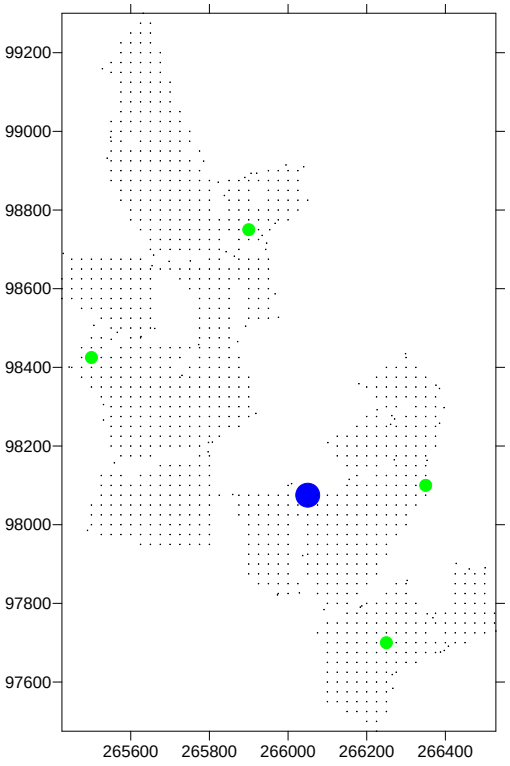
(g) Stratiomyidae



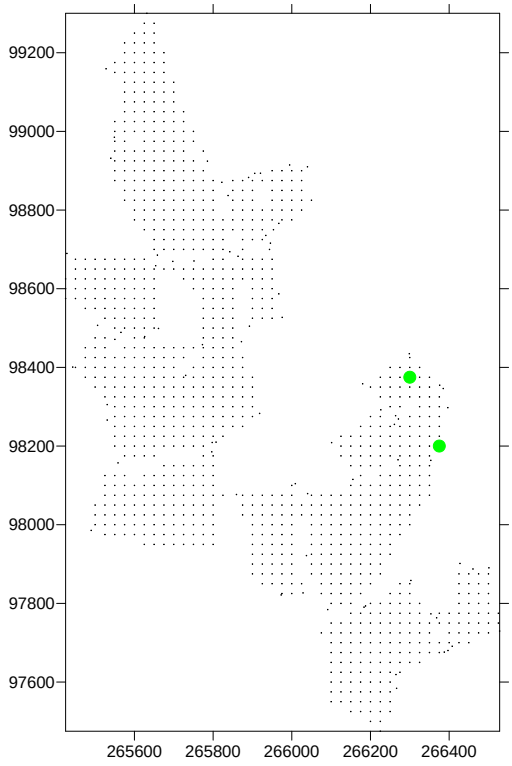
(h) Adult Carabidae



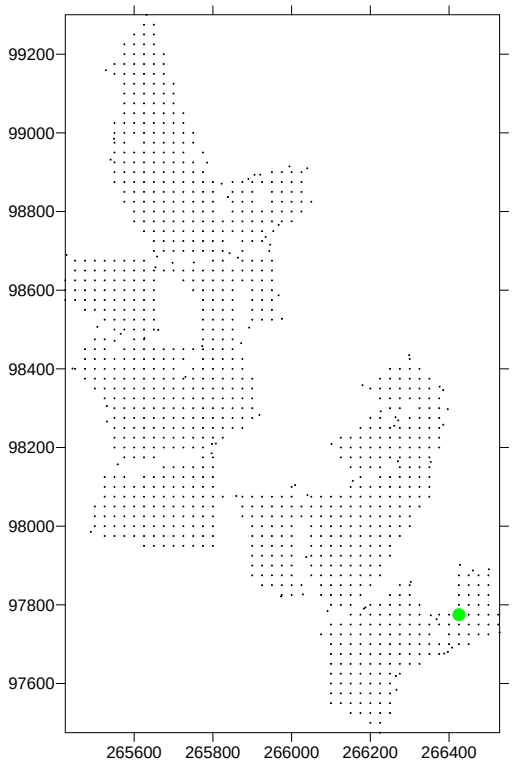
(i) Carabidae larvae



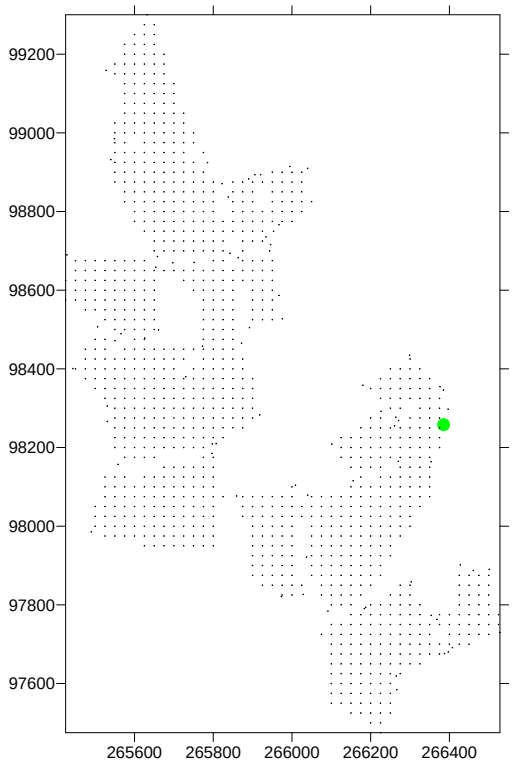
(j) Cantharidae



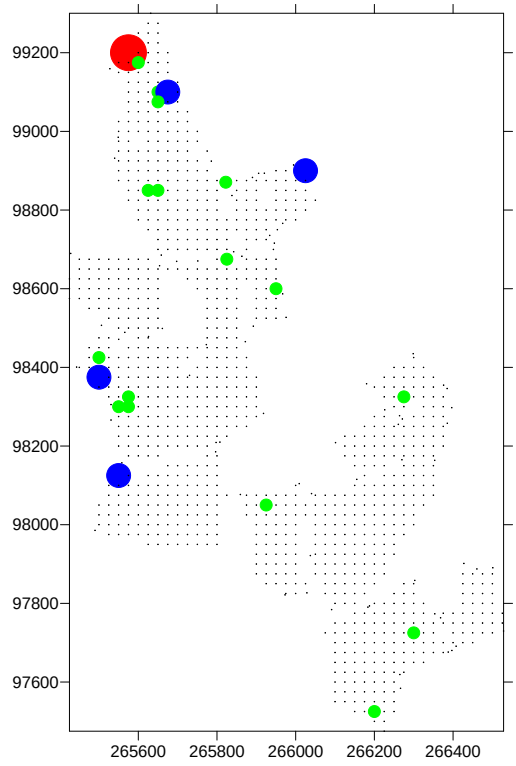
(k) Elateridae



(l) Noctuidae



(m) Dolichopodidae (Type B)



2.4.6 Variance/mean ratio

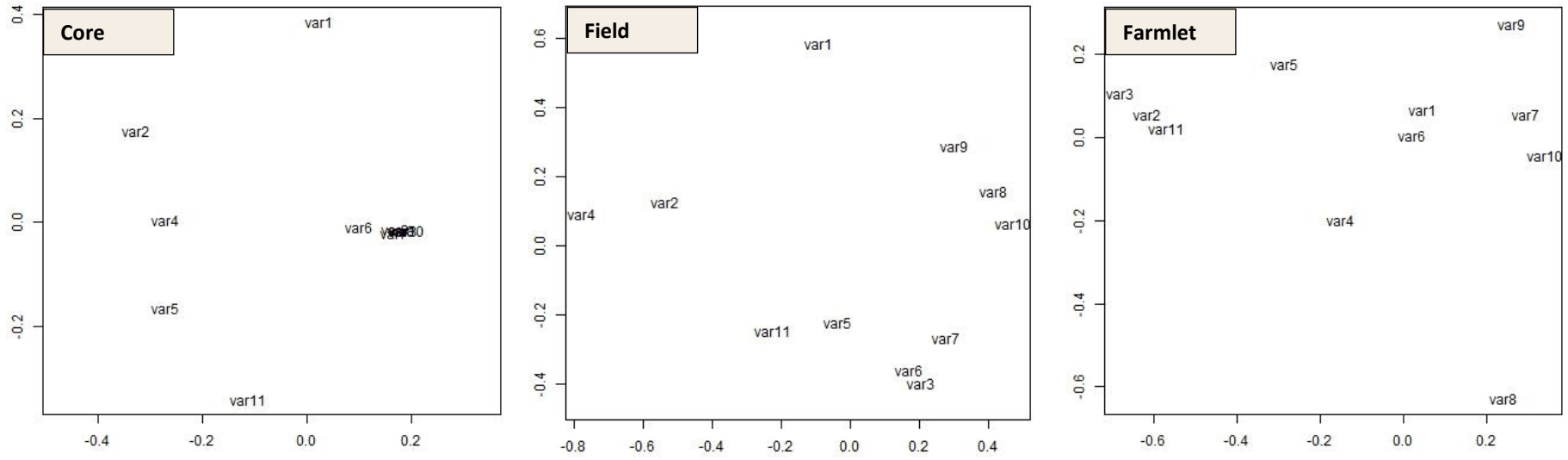
The variance / mean ratio showed differences between the distribution of taxa at each scale. However, for some taxa there were insufficient numbers wich were recovered in this study such as the larvae of Elateridae, Noctuidae, Cantharidae and both adult and larval Carabidae;therefore, the the variance to mean ratios may not a represent the complete biological mechanisms for these low numbered taxa (Table 2.5). The families Tipulidae and Muscidae were randomly distributed at the core scale but had an aggregated distribution pattern at both field and the farmlet scale respectively. The family Chrysomelidae had an aggregated distribution pattern at the core and the field scale but were randomly distributed at the farmlet scale. The adult Carabidae had a random distribution pattern at the core and the field scale but were agregated at the farmlet scale. The Carabidae larvae were also agregated at field and farmlet scale but they were randomley distributed at the core scale.The families Chironomidae, Psychodidae, Dolichopodidae (Type B), Bibionidae and Stratiomyidae were agregated all scales whereas Cantharidae, Elateridae and Noctuidae were randomely distributed at all scales (Table 2.5).

Table 2.5 The variance/mean ratio for the taxa recovered in 2013 at the farm platform at three different spatial scales (farmlets, field, and core scale).

Taxa	Farmlet	Field	Core
Tipulidae	10.6	3.3	1.1
Chironomidae	24.7	19.2	2.3
Psychodidae	21.8	27.6	29.0
Dolichopodidae (Type B)	5.1	2.4	1.5
Bibionidae	5.2	7.8	3.0
Chrysomelidae	1.1	2.5	1.6
Stratiomyidae	2.5	2.7	1.6
Carabidae (Adults)	3.0	1.4	1.0
Carabidae (Larvae)	1.5	1.7	1.3
Cantharidae	0.5	0.9	1.0
Elateridae	0.8	1.0	1.0
Noctuidae	0.8	1.0	1.0
Muscidae	6.4	4.8	1.2

2.4.7 Non-metric Multi Dimensional Scaling (NMDS)

The NMDS revealed that the species interactions vary across the three sampling scales (core, field and farmlet). For the core scale, the families Psychodidae, Chrysomelidae, Stratiomyidae, Carabidae (Adults and larvae), Cantharidae and Muscidae larvae were found in close proximity whereas, the families Tipulidae, Chironomidae, Dolichopodidae (Type B) and Bibionidae larvae were dissociated from each other (Figure 2.5 core). For the field scale, the families Chrysomelidae, Psychodidae, Stratiomyidae were found in close distances similar to Carabidae (Adults and larvae) and Cantharidae. There were also slight associations between Bibionidae and Muscidae larvae on one side and the Chironomidae and Dolichopodidae (Type B) on the other side with the dissociation of the Tipulidae larvae from all the taxa (Figure 2.5 field). For the farmlet scale, the families Chironomidae, Psychodidae and Muscidae were found in close distances. The Tipulidae and Chrysomelidae larvae were also found in close distances similar to Stratiomyidae and Cantharidae. The families Dolichopodidae (Type B), Bibionidae and Carabidae larvae were dissociated from the rest of taxa at the farm scale (Figure 2.5 farmlet).



Dimensions= 2 Stress= 0.1140296 Dimensions= 2 Stress= 0.0870999 Dimensions=2 Stress= 0

Figure 2.5 NMDS ordination biplot (axes 1 and 2) for core, field and farmlet scale. The close distances between taxa elucidate coexisted species. The insect families are: var1=Tipulidae, var 2= Chironomidae, var 3= Psychodidae, var 4= Dolichopodidae (Type B), var 5= Bibionidae, var 6= Chrysomelidae, var 7=, Stratiomyidae, var 8= Carabidae-adults, var 9=, Carabidae larvae var 10= Cantharidae, var 11= Muscidae.

2.4.8 Deviance partitioning

The percentage of deviance explained through deviance partitioning varied for the species data (precence/absence) between three groups of explanatory variables (Scale, Space and Biotic) which ranged between 10% (Leatherjackets) to 47% (Cantharidae) and the majority of the variations are shown in table 2.6. Scale comprised the most explained deviance for the taxa which were between 8% (Leatherjackets) to 50% (Chironomidae) in comparison with biotic and space. The combined explained deviance for the biotic and scale variables was relatively greater than the scale and space variables and both explained more deviance than biotic and space which was relatively small. Space and farmlet scale explained an equal amount of deviance for leatherjackets and Chrysomelidae (1% and 2% respectively) suggesting that these factors dot not have a considerable influence on these species. The importance of scale (Table 2.6) showed that all the taxa are more influenced by the field scale than the farmlet scale suggesting that farmlet scale does not considerably affect the distribution of taxa in comparison with the field scale. The core scale is not included here due to the high number of zero counts (cores with no taxa). Only 201 cores out of 1130 contained three different taxa (Diptera, Coleptera and Lepidoptera) and they were not significantly different from the predicted number of occasions of co-occurrence calculated by chi square test (x^2= 4, df= 51 , p>0.05) suggesting that taxa are distributed randomly at this scale.

Table 2.6 The percentage of explained deviance explained through deviance partitioning in the dependent variables (species presence/absence) between three groups of explanatory variables (location, space and biotic), their combinations, and the deviance unexplained by the variables in this study for each taxa.

Taxa	Biotic	Scale (All)	Scale (Farm)	Scale (Field)	Space	Biotic and scale	Biotic and space	Scale and space	All variables	Unexplained
Tipulidae	3	8	1	7	1	10	4	7	10	90
Chironomidae	11	50	20	30	16	37	25	30	37	63
Psychodidae	2	43	14	29	7	33	10	36	42	58
Dolichopodidae (Type B)	12	15	3	12	4	22	15	14	24	76
Bibionidae	10	35	4	31	7	38	16	31	38	62
Chrysomelidae	18	23	2	21	2	33	18	22	34	66
Stratiomyidae	7	25	4	21	3	33	13	23	34	66
Carabidae (Adults)	3	47	15	32	2	34	5	34	36	64
Carabidae (Larvae)	9	29	3	26	1	35	11	27	35	65
Cantharidae	2	45	5	40	36	41	38	47	47	53
Muscidae	5	41	14	27	17	34	24	28	34	66

2.5 Discussion

2.5.1 The abundance of taxa

Unlike other reports (Blackshaw, 1984; Johnson & Murray, 2008), the numbers of taxa recovered for both years' data were low and few insects were recovered (Tables 2.1 and 2.4). The results of both years' data showed seasonal variations in the abundance and composition of taxa in each year of sampling. The family Tipulidae was the most abundant taxa recovered in both years of the investigation. Previous studies in the UK indicate that the family Tipulidae can occur in large populations, are the biggest dipteran family with more than 300 species and that their occurrence is higher in parts of northern and south eastern England (Figure 1.1) (NBN, 2013; Smith, 1989). In grassland soils, leatherjackets have been recorded previously in relatively high abundances in comparison with other taxa as found in this study (Buckle, 1923; Staley et al., 2007). Blackshaw & Petrovskii (2007) showed that the populations of *T*. *paludosa* are regulated by density dependent feedback at regional scales. Recent studies by Bearup et al. (2013) have shown similar results at the field scale.

The abundance of Sciaridae has also been recorded as the second most numerous dipteran families in grasslands after Bibionidae (Benefer et *al.*, 2010). However, no Bibionidae larvae were detected in 2012 in comparison with 2013. D' Arcy - Burt and Blackshaw (1991) reported that bibionid larvae occur only sporadically in grassland soils. Low numbers of Bibionidae were recovered in this study with a low population density (19,100ha^{-1}) in comparison with other reports such as 230,000ha^{-1} for the genus *Bibio johannis* and 120,000 for *Dilophus*

febrilis (Benefer *et a*l., 2010). Blackshaw and D'Arcy-Burt (1993) concluded that the sample size of Bibionidae is considerably determined by sampling process resources. Despite this, sampling periods and the biology may be another factor of determining Bibionid populations; for *B. johannis,* for instance, growth is slow during late summer and early autumn but high from October to January (Blackshaw & D'Arcy-Burt, 1992).

The abundance of the families Muscidae, Chironomidae and Dolichopodidae (Type B) were higher in 2013 than in 2012 as populations increased by 2300 ha^{-1}, 41700 ha^{-1} and 15800 ha^{-1} respectively. In contrast, the abundance of the families Stratiomyidae and Carabidae (adults and larvae) were lower in 2013 than in 2012 and numbers decreased by 5700 ha^{-1}, 10100 ha^{-1} and 7780 ha^{-1} respectively (Tables 2.1 and 2.4).

The composition of Noctuidae and Elateridae larvae were low and each comprised the lowest number of all taxa recovered (Table 2.4). Curry (1994) reported that the populations of lepidopteran larvae are known to be low generally in grassland ecosystems as found for Noctuidae larvae in this study. On the other hand, only one Elateridae larva was recovered in this study in comparison with other studies and this could be related to the abiotic factors mainly temperature as it is known to alter the seasonal movement (horizontal and vertical) of wireworms in the soil (Fisher, Keaster & Fairchild, 1975; Lafrance, 1968) (see also Chapter 1 for further details).

2.5.2 Species interactions

The interspecific interactions between species have become a controversial and unresolved issue in studies of community ecology particularly for phytophagous insects (Kaplan & Denno, 2007). The interspecific competition over a habitat is known as one of the primary biotic factors influencing the abundance, distribution pattern and diversity of ecological communities (Begon, Townsend & Harper, 2009) but these interactions vary between sampling scales, as at large scales this is determined by species specific requirements or environmental factors whereas at smaller scales, competition and predation may have more impact (Wiens, 1989).

The species interactions varied across sampling scales (Core, field and farmlet) for both years of study (Figures 2.3 and 2.5). For example, the families Tipulidae and Dolichopodidae (Type B) were found in close proximity at the core scale in 2012 but they were dissociated at the same scale in 2013. This could possibly be due to biotic factors such as reproductive capacity that enables many insects at higher populations to colonize new habitats and exploit favourable conditions or new resources quickly whereas at lower populations, the ability to colonize new patches is limited by their small size, short life span, and dependence on chemical communication to find mates (Schowalter, 2011). Blackshaw and Moore (2012) also showed that biotic factors influence leatherjacket numbers when they reach the fourth larval instars as older leatherjackets move to sites where favourable soil moisture are available with implication of changing spatial pattern. Here, the Dolichopodidae (Type B) larvae were found in higher populations in 2013 than in 2012 thus this could explain their dissociations from Tipulidae at the

core scale in 2013 through a greater ability to colonize new patches (Figures 2.3 and 2.5). In addition, at larger scales the distribution is more determined by species specific habitat requirement or environmental factors (Wiens, 1989). This could be the reason for the dissociation of the Dolichopododae larvae from Tipulidae in both years at the field and farmlet scale (Figures 2.3 and 2.5). For the Dolichopodidae larvae (Type B), they were mostly found in field margins in both years (Figures 2.2h and 2.4m). In addition, the Dolichopodidae (Type A) were also found mostly in field margins in 2012 (Figure 2.2a), thus this could be their favoured habitat in comparison with Tipulidae which were found throughout the fields (Figures 2.2a and 2.4a). The close associations between Muscidae larvae with Stratoimyidae and Carabidae (adults and larvae) at the core scale and their dissociations at the field and farm scale (Figure 2.5) could also be interpreted as their distribution at larger scales being determined by species specific habitat requirement and environmental factors (Wiens, 1989).

Despite being common in grassland soils (Benefer *et al*., 2012), the families Elateridae and Bibionidae were found only in small numbers in 2013 whereas Sciaridae larvae were found with low numbers only in 2012 survey. The reason for not recovering Elateridae and Bibionidae larvae in 2012 and their occurrence in low numbers in 2013 could be related to their biology and/or environmental factors that may affect their occurrence such as temperature and moisture which are not investigated in this study. It has been well known that the temperature could affect the activity of wireworms with in the soil as dormancy occurs when temperature decreases and extreme decreases causes mortality (Campbell,

1937). Lafrance (1968) found that decreases in temperature to 19.4 °C affect the vertical movement of wireworms and larvae moves deeper into the soil with only few numbers remaining on the top layer of the soil. The occurrence of Bibionidae larvae in low numbers in 2013 could refer to its biology as Bibionidae larvae are known to occur only sporadically in grassland soils but can have considerable damage when they reach high numbers (D'Arcy-Burt & Blackshaw, 1991).

The other taxa such as Dolichopodidata (Type A), Scatopsidae, Anthomyiidae and unknown Coleoptera were found only in 2012 survey whereas the families Psychodidae, Chrysomelidae, Cantharidae, and Noctuidae were found only in 2013 survey. All These taxa were found only in small numbers in comparison with Tipulidae. Curry (1994) reported that the dipteran families Psychodidae, Scatopsidae, Dolichopodidae and Anthomyiidae are found mostly in animal dung as their favoured habitat whereas the beetle family Cantharidae occur mostly as predators in grassland soils. Only one Noctuid larva were recovered in 2013 survey as Curry (1994) has reported that the population of lepidopteran larvae are low generally in grassland soils. The low numbers of Chrysomelidae larvae found only in 2013 could be related the effect of abiotic factors for example, Linzmeier and Ribeiro-Costa (2013) found that the photoperiod in conjunction with temperature and relative humidity (R.H) has a significant effect on the seasonal abundance of Crysomelidae.

2.5.3 Deviance partitioning

The effect of scale, space and biotic variables on species distribution using deviance partitioning showed scale as the most important of the three factors affecting species distributions in comparison with space and biotic variables for both years, comprising 8% to 50 % of total explained deviance (Tables 2.3 and 2.6) However, for the Tipulidae larvae, scale explained only a little amount of deviance for both years (11% and 8% for 2012 and 2013) data respectively (Tables 2.3 and 2.6). This is in agreement with previous studies by Benefer *et al.*, (2010) who indicated 10 % of the explained deviance by scale and 88% of unexplained deviance for leatherjackets in grassland; indicating that scale does not considerably affect the distribution of grassland leatherjackets and there are other unmeasured factors which could play an important role in the distribution of grassland leatherjackets (Benefer *et al.*, 2010). Recently, the spatial distribution of leatherjackets has been linked to habitat quality and is mainly explained by soil moisture (Petersen, Seto & Peck, 2013). In addition, the effect of biotic and space factors were nearly similar for 2012 data but space showed the least explained deviance for almost all taxa in 2013. The combined effect of biotic and scale variables were more for almost all taxa than the combined scale and space and biotic and space variables respectively for both years' surveys with only 10% of explained deviance for leatherjackets in both years indicating that even the combined effect of these factors does not considerably influence leatherjacket distributions (Tables 2.3 and 2.6).

A substantial conclusion of this study is that the distribution of each individual taxon varied generally and is probably linked to different scales and species-specific factors. For example, the most explained deviance was provided by all scales (farm and field). The Space factor explained an equal amount of deviance for Dolichopodidae (Type B) as the biotic but not for Dolichopodidae (Type A), demonstrating possible diversity in the biology of these two species. This may refer to biological mechanisms rather than environmental factors for example, growth, predation, competition, and social aggregation (Borcard, Legendre & Drapeau, 1992). Nonetheless, only a little amount of deviance was explained for leatherjackets by scale, biotic and spatial variables with 90% of deviance not explained adequately (Table 2.3) indicating that these factors (variables) are ineffective in explaining leatherjacket distributions – as also found by Benefer *et al.*, (2010). The similarity of deviance explained by Space and Biotic separately for Carabidae larvae and Dolichopodidae (Type B) suggests that the factors determining reproduction or response to biotic mechanisms such as cannibalism (Blackshaw & Coll, 1999; Blackshaw & Petrovskii, 2007) has less impact than the impact of scale. These findings could highlight some of the most important factors affecting species distributions in grassland soils and their interactions. Using techniques that could reliably identify the taxa into species level may help a better understanding of species distributions and the factors driving their distributions.

Chapter 3

Molecular Identification and phylogeny of leatherjackets (Diptera:Tipulidae) in agricultural grassland

Chapter 3: Molecular identification and phylogeny of leatherjackets (Diptera:Tipulidae) in agricultural grassland

3.1 Introduction

Recently, the use of molecular diagnostic techniques has become an important tool for the identification of many organisms and the study of genetic populations. These have been mainly divided into three main areas of study: protein electrophoresis, DNA sequencing and fragment analysis of DNA (Lindroth, 2007). The DNA sequencing methods have recently played a role in the genetic identification of various cryptic agricultural pests for those which their identification depending on morphology represents a challenge (Bickford *et al*., 2007) such as cryptic wireworm species within the genus *Agriotes* (Ellis *et al.*, 2009), *Bemisia tabaci* (Dickey *et al.*, 2013; Esterhuizen *et al*., 2013), aphids (Piffaretti *et al.*, 2013; Rebijith *et al*., 2013), cryptic apple and strawberry maggots (Green *et al*., 2013) and spider mites (Matsuda *et al*., 2013).

The Cytochrome c oxidase subunit I (COI) is a mitochondrial protein coding genome that is often used as a DNA barcode to identify animal species which have the ability to diagnose closely related species in certain taxonomic groups (Hebert, Ratnasingham & de Waard, 2003). It has been reported to be an effective gene for the identification system of animal life which would window the 10 million animal species down to the generic level with less than 10 species on average and can race the last digit of animal diversity through giving a 99.9999% resolution of animal diversity (Hebert, Ratnasingham & de Waard, 2003). COI sequences have been widely used in many entomological studies for genetic identification, phylogenetics and population studies of agricultural pests (e.g. Barr

et al., 2012; Boehme *et al*., 2010; Jung, Duwal & Lee, 2011; Park *et al*., 2011a; Park *et al*., 2011b) because this is supposed to be among the most conservative protein-coding genes of the animal mitochondrial genome (Vrijenhoek, 1994). There have been various studies on the use of COI DNA barcoding for the identification of many insect species for example, true bugs (Jung, Duwal & Lee, 2011; Lis, Lis & Ziaja, 2013), true flies (Boehme, Amendt & Zehner, 2012; Nelson, Wallman & Dowton, 2007; Shin *et al*., 2013) and Lepidoptera (Ashfaq *et al*., 2013; Hajibabaei *et al*., 2006). Despite that, studies on the use of COI DNA barcodes for the identification of soil insects, in particular in grasslands, is limited (e.g. Staudacher et al., 2011a) and few have investigated the genetic identification of grassland Tipulidae namely *Tipula paludosa* and *oleracea* which has an economic importance in agricultural grassland (Humphreys *et al.*, 1993; Rao et al., 2006). In addition, knowledge of the proportion of both species to each other is lacking in agricultural grassland in south west England.

In the UK, these two species are endemic pests of agriculture (Blackshaw & Coll, 1999) and damage by leatherjackets in grassland has been reported to be significant yearly in Northern Ireland (Blackshaw, 1985) and south west Scotland (Johnson & Murray, 2008). Larvae of the two species are known to feed and damage a variety of crops (see chapter 1).

Adults of both species can be separated depending on several morphological characters such the distance between compound eyes on the ventral side of the head capsule which is broader in *T. paludosa* in comparison with *T.oleracea* in both males and females, wing length in females of both species which is shorter

than the abdomen for *T. paludosa* and longer for *T. oleracea,* and the shape of male genitalia varies clearly between the two species (Hoebeke & Klass, 2005).

Despite the differences in the morphology of adults, larvae of the two species are extremely similar and their identification represents a challenge (Rao *et al*, 2006). The Identification of larvae is critical in terms of pest management strategies before making any decision. For example, in the case of applying biological control agents as an alternative to pesticides, the two common species (*T. paludosa* and *oleracea*) cannot be distinguished in the soil due to the differences in their life cycle, feeding pattern and the damage period throughout the year thus leading to misusing biological control agents if the two species is not distinguished. Previous studies have suggested that microscopic hair patterns can distinguish between larvae of the two species (Brindle, 1959) however, this is still not known to be a reliable character (Gelhaus, 2005). Other studies have used isoelectric focusing to differentiate between larvae of the two species (Humphreys *et al.*, 1993) but this does not separate between these and other species of *Tipula* (Blackshaw & Coll, 1999). Recently, the development of molecular methods is one way to differentiate between larvae of the two species using methods such as DNA barcoding (Hebert, Cywinska & Ball, 2003) through amplification of mitochondrial cytochrome c oxidase subunit I (COI) using DNA primers (Rao *et al.*, 2006). In the current study we aimed to develop a molecular method using universal primers (Folmer primers with degenerate bases) to identify subterranean insects in agricultural grassland and study their genetic diversity.

The specific objectives of this study were to:

1- Identify larvae of *T. paludosa* and *T. oleracea* in agricultural grassland at the North Wyke Farm Platform in Devon.
2- Investigate the intra and inter specific genetic variability among and between the two species.
3- Construct a phylogenetic relationship between *T. paludosa* and *T. oleracea* adults and larvae.

3.2 Materials and Methods

3.2.1 DNA extraction and genetic Identification of Tipulidae

Adults of both species (*T. paludosa* and *T. oleracea*) were collected from agricultural grassland at Rothamsted, North Wyke farm platform using hand nets and light traps. Both species were separated depending on morphological characters (Blackshaw & Coll, 1999; Hoebeke & Klass, 2005). Samples were frozen at -20°C for later DNA extraction.

Tipula larvae recovered in the surveys described in Chapter 2 were used as source material. The first year's larvae (106 samples) were kept at 70% ethanol for later DNA extraction and identification but the second year's larvae (117 samples) were kept at -20^{o} for later DNA extraction and other analysis.

DNA was extracted following the Spin-column protocol for purification of total DNA from animal tissues (DNeasy® Tissue Kit - Qiagen, Hilden, Germany). For both adult Tipulids and larvae, one of the central abdominal segments (depending on size) were cut into small pieces using a sterilized blade and added to 1.5 ml

Microcenterfuge tubes. 180 µl of ATL buffer was pipetted to each sample and then 20 µl of proteinase K into the tubes and incubated at 56°C until the tissue was completely digested. Samples were vortexed for 15s. then 200 µl of AL buffer was added to each sample and mixed thoroughly by vortexing. 200 µl of ethanol (96-100%) was added to each sample and mixed again thoroughly by vortexing. The mixture obtained from each sample was pipetted (including any precipitate) into DNeasy Mini spin columns and placed in a 2 ml collection tube. Samples were centrifuged at 8000 rpm for 1 min. The flow-through and the collection tubes were discarded and the DNeasy Mini spin columns were placed in new 2 ml collection tubes. 500 µl of buffer AW1 was added to each sample and centrifuged for 1 min at 8000 rpm. The flow-through and the collection tubes were again discarded. The DNeasy Mini spin columns were placed in new 2 ml collection tubes and 500 µl of AW2 buffer was added to each sample and centrifuged for 3 min at 14000 rpm to dry the DNeasy membrane. The flow-through and the collection tubes were discarded. The DNeasy Mini spin columns were placed in clean 1.5 ml microcentrifuge tubes and 200 µl of AE buffer were added directly onto the DNeasy membrane. Samples were incubated at room temperature for 1 min then centrifuged for 1 min at 8000 rpm to elute, and preserved in the fridge for later PCR applications.

PCR was performed at the Plymouth molecular lab using a Qiagen Taq PCR Core kit (Table 3.1). Individual PCR tubes were labelled with the sample name. 1ul of each sample (adult Tipulidae) was added to the labelled tubes. 1µl of COI forward primer (COIF) (5'-TTTCAACAAATCATAARGAYATYGG-3'), 1µl

labelled reverse primer (COIR) (5'- TAAACTTCNGGRTGNCCAAAAAATCA-3'). 2 µl dNTP, 3 µl MgCl2, 2.5 µl 10X buffer, 0.1 µl Taq and 14.4 µl DNA grade H_20 was added for a total 25 µl reaction for each sample (keeping the Taq in the freezer until it was ready to use). The reagents were well mixed by pipetting the mixture up and down several times. The closed PCR tubes were placed into the PCR machine and the PCR cycle steps (Table 3.2) were selected which took approximately 3 hours to run. The tubes were removed and kept in the fridge (short-term use).

For the larvae, problems were encountered with PCR amplifications. When the same (adult) PCR protocol was applied to the larvae there were no PCR products in comparison with adults possibly due to PCR inhibitors (Braid, Daniels & Kitts, 2003; Juen & Traugott, 2006). Different methods were tested such as changing the annealing temperature and PCR cycles (Rychlik, Spencer & Rhoads, 1990) and using Q solution which is used for PCR systems that do not work well under standard conditions (Taq PCR Handbook) but no results were obtained when these methods tested. We were finally able to overcome PCR inhibitors using 1µl of Bovine Serum Albumin (BSA) (Juen & Traugott, 2006) which was added to 24µl of PCR mix (Table 3.1).

Table 3.1 Qiagen Taq PCR Core kit reagents with their volume and concentrations used to amplify the COI region of Tipulidae mitochondrial DNA.

***** Adult Tipulidae, ** Tipulidae Larvae.

1 sample	*Volume/µl	**Volume/µl	Concentration	Final concentration
PCR buffer	2.5	2.5	10_X	1_X
MgCl2	3	3	25mM	3mM
dNTPs	2	2	10mM of each	200µM
FP	1	1	25µM	1µM
RP	1	1	25µM	1µM
BSA	-	1	-	-
Taq	0.1	0.1	0.5 units	0.5 units
DNA grade H2O	14.4	13.4	-	-
DNA	1	1	-	-
Total	25	25	-	-

Table 3.2 PCR cycles used to amplify the mitochondrial DNA of the COI gene in Tipulidae (Aadults and larvae).

Step	Time/min	Temp/°C	Number of cycles
Initial denaturation	3	94	
Denaturation	1	94	
Annealing	30s	52	35 cycles
Extension	1	72	
Final extension	10	72	

PCR samples were run on 2% agarose gel and checked with a gel doc imaging system (Plymouth molecular lab). There were PCR products (Figure 3.1) for about 710-bp fragments of the mitochondrial cytochrome c oxidase subunit I gene (COI). PCR products were then cleaned up using an exo-sap cycle in PCR tubes in the PCR machine. 2 µl of the exo-sap reagent was added to each 20 µl of the PCR product and then incubated in the PCR machine for 1 hour at 37°C followed by 15 minutes at 80°C (Table 3.3).

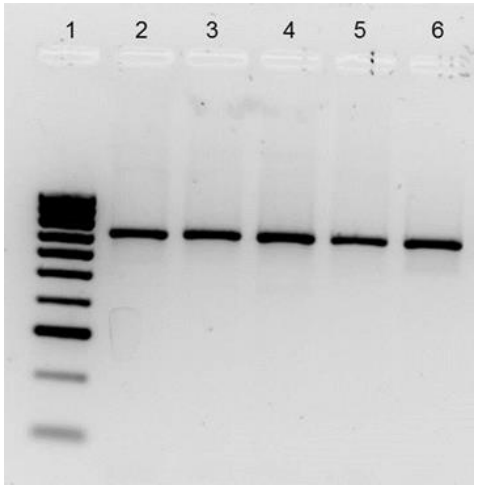


Figure 3.1 agarose gel of PCR products from COI gen of Tipulidae. Lane 1 is the ladder, the lanes 2- 5 are T. paludosa larvae which have been amplified using BSA and lane 6 is an adult T. paludosa (control with no BSA).

Table 3.3 The Exo-Sap cycle materials used for cleaning the amplified DNA prior to sequencing.

1sample	Volume/µl	Concentration
Exo	0.5	10 units
Sap	1	1 unit
DNA grade H_2O	0.5	-
Total	2*	-

*** Added to 20µl PCR product**

3.2.2 Sequence data and phylogenetic analysis

The successful and cleaned PCR products were sequenced by Macrogen Incorporation (South Korea). Due to the limited materials for DNA extraction and time of study period, only 182 samples of Tipulidae (25 adults and 157 larvae) were sequenced using COI universal primers (Vrijenhoek, 1994). The obtained

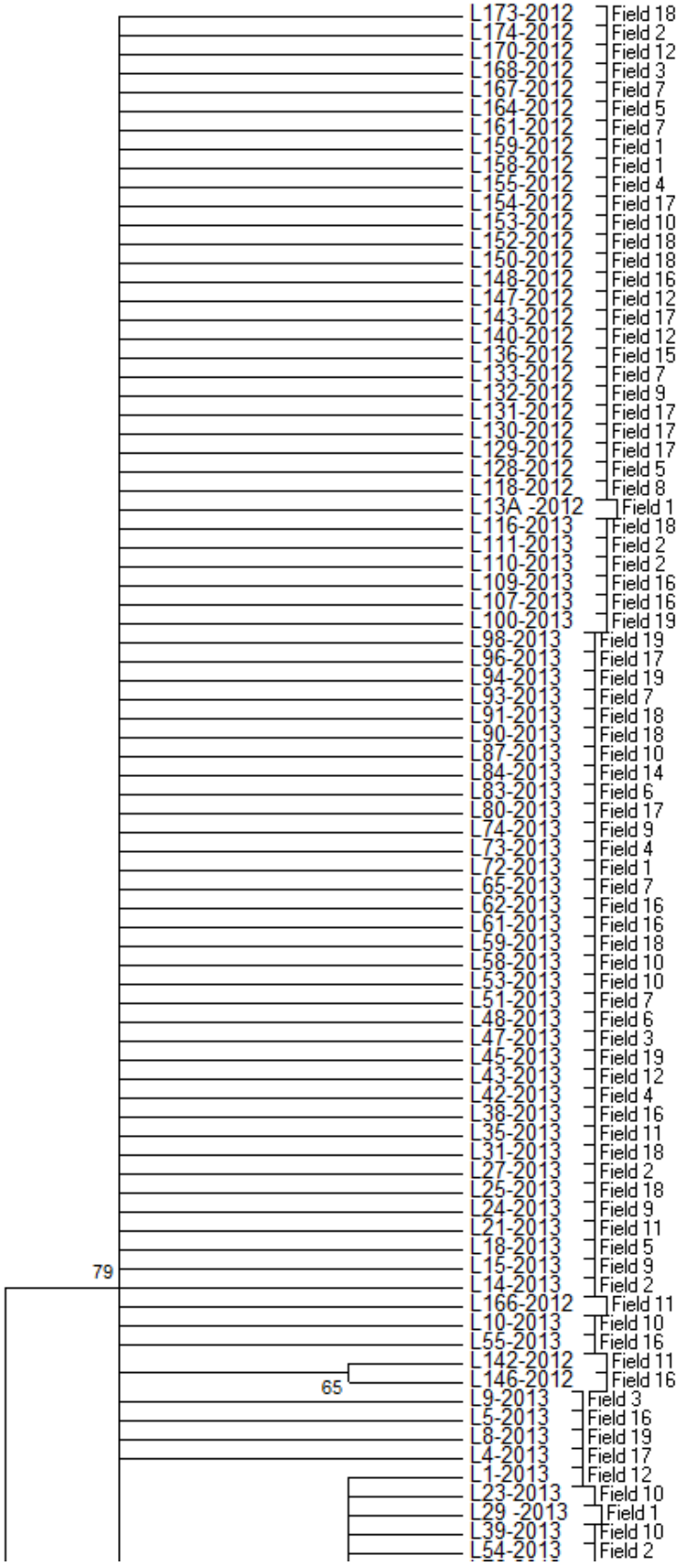
sequences were edited using Bio Edit – Version 7.1.9.0 (Hall, 1999) then compared with the Barcode of Life Data Systems (BOLD Systems V3 - http://www.boldsystems.org/) for species identifications. In total, 162 specimens of *T. paludosa* (19 adults and 143 larvae) and 12 species of *T*. *oleracea* (6 adults and 6 larvae) were identified based on their specific sequences. For the remaining eight samples, their sequences were messy and did not match clearly to either*T. paludosa* or *T. oleracea* (for example, L70 only matched 85% to *T*. *paludosa*) possibly due to poor DNA quality and/or extraction methods. These samples were eliminated from the analysis.

The sequences were aligned by ClustalW in MEGA 5.1(Tamura *et al*., 2011). Of about 700 bases, only 462 characters were analysed because of the short sequences of some species when aligned, or gaps. A phylogenetic tree was constructed in MEGA 5.1 for the larval *T. paludosa* and *oleracea* data grouped by fields and year using Neighbour Joining tree (Figure 3.1) with P-distance as a measure of genetic distance and tested by bootstrap with 1000 replicates. The complete deletion was chosen for missing data and gaps. The transitions and transversions substitution and homogenous pattern among linages were chosen with uniform rates among sites. *Tipula recondita* Pilipenko & Salmela, sp. n. specimen was designated as the outgroup for the taxa and its sequence downloaded from the BOLD data base (http://www.boldsystems.org).

3.3 Results

The tree placed both *T*. *paludosa* and *T*. *oleracea* in two separate clades with a bootstrap support of 77 % and they had P = 0.06 nucleotide divergence on average indicating these two different species as sister groups to each other. The average nucleotide divergence within both species including adults and larvae was between P = 0.02 for *T*. *paludosa* (both adults and larvae) and P = 0.01 and 0.02 for both adult and larval *T*. *oleracea* respectively. For *T*. *oleracea* larvae, the tree places both L19 and L104 in one clade with a 65% bootstrap value indicating the genetic similarity of the two species which both belong to field 1 samples collected in 2013. Similarly, L20 and L85 were closely related and placed in a separate clade with 68% bootstrap value belonging to the fields 17 and 6 respectively. These samples with L37 were placed in one clade with 59% bootstrap support and composed a basal clade for L103 with 99% bootstrap support indicating a very strong similarity and relationship within these species of *T*. *oleracea* collected from different fields in 2013.

For *T*. *paludosa* larvae, there were samples collected in both years in 2012 and 2013 survey and analysed in the tree based on both year and field groupings. L160, L149 (field 7), L97 (field 5), L46 (field 10), and L3 (field 4) were closely related and placed in a separate clade with bootstrap 89% support and were strongly placed with L34 (field 4). L64 (field 2) was placed as a basal node for other clades within *T*. *paludosa* larvae with 82% bootstrap value. There were intraspecific genetic variations within *T*. *paludosa* species collected at each field in both years experiments and these species were clustered in different clades based on their location within fields and the time of year collected (Figure 3.2).


L173-2012 Field 18
L174-2012 Field 2
L170-2012 Field 12
L168-2012 Field 3
L167-2012 Field 7
L164-2012 Field 5
L161-2012 Field 7
L159-2012 Field 1
L158-2012 Field 1
L155-2012 Field 4
L154-2012 Field 17
L153-2012 Field 10
L152-2012 Field 18
L150-2012 Field 18
L148-2012 Field 16
L147-2012 Field 12
L143-2012 Field 17
L140-2012 Field 12
L136-2012 Field 15
L133-2012 Field 7
L132-2012 Field 9
L131-2012 Field 17
L130-2012 Field 17
L129-2012 Field 17
L128-2012 Field 5
L118-2012 Field 8
L13A -2012 Field 1
L116-2013 Field 18
L111-2013 Field 2
L110-2013 Field 2
L109-2013 Field 16
L107-2013 Field 16
L100-2013 Field 19
L98-2013 Field 19
L96-2013 Field 17
L94-2013 Field 19
L93-2013 Field 7
L91-2013 Field 18
L90-2013 Field 18
L87-2013 Field 10
L84-2013 Field 14
L83-2013 Field 6
L80-2013 Field 17
L74-2013 Field 9
L73-2013 Field 4
L72-2013 Field 1
L65-2013 Field 7
L62-2013 Field 16
L61-2013 Field 16
L59-2013 Field 18
L58-2013 Field 10
L53-2013 Field 10
L51-2013 Field 7
L48-2013 Field 6
L47-2013 Field 3
L45-2013 Field 19
L43-2013 Field 12
L42-2013 Field 4
L38-2013 Field 16
L35-2013 Field 11
L31-2013 Field 18
L27-2013 Field 2
L25-2013 Field 18
L24-2013 Field 9
L21-2013 Field 11
L18-2013 Field 5
79
L15-2013 Field 9
L14-2013 Field 2
L166-2012 Field 11
L10-2013 Field 10
L55-2013 Field 16
L142-2012 Field 11
65
L146-2012 Field 16
L9-2013 Field 3
L5-2013 Field 16
L8-2013 Field 19
L4-2013 Field 17
L1-2013 Field 12
L23-2013 Field 10
L29 -2013 Field 1
L39-2013 Field 10
L54-2013 Field 2

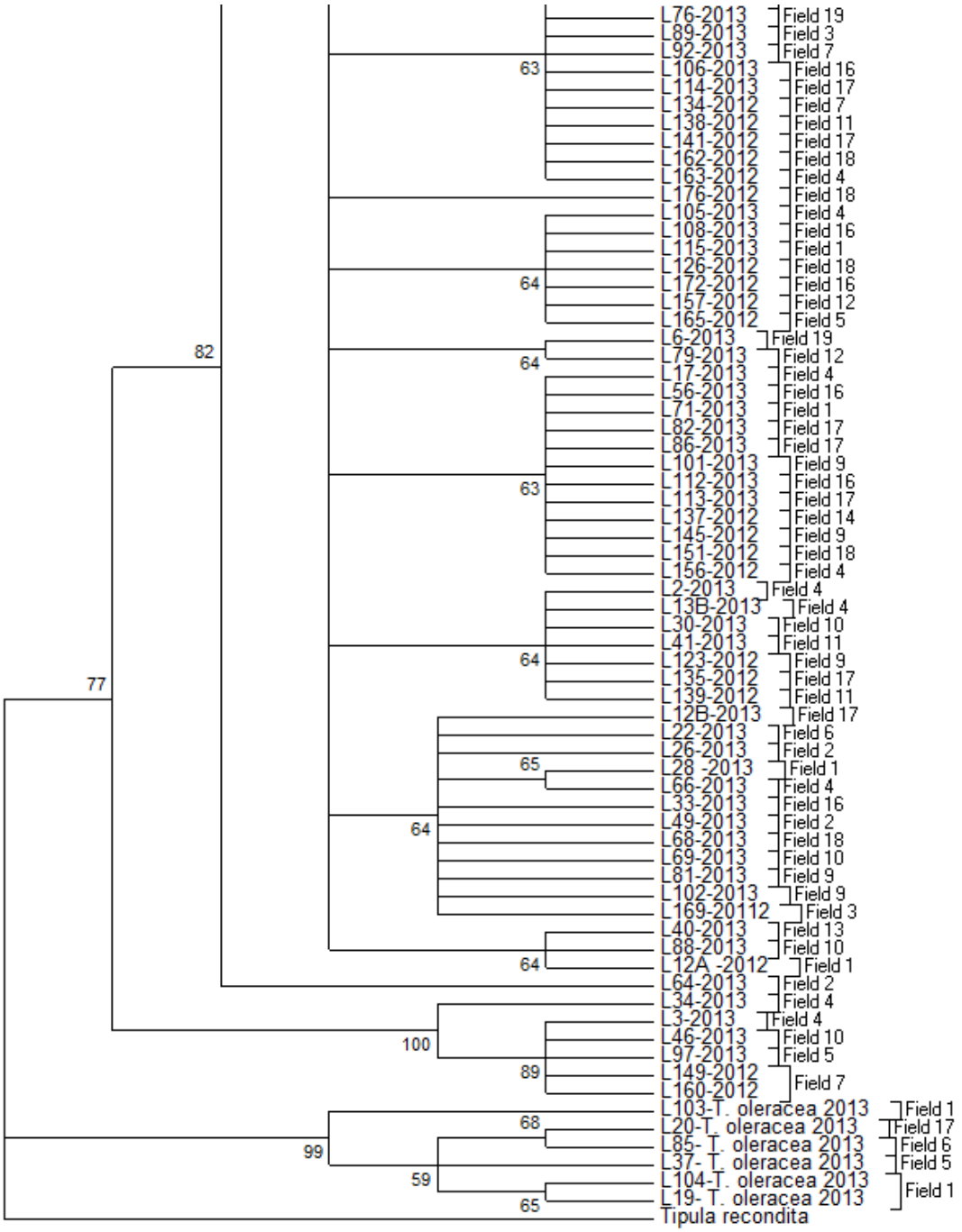


Figure 3.2 Neighbour joining tree using p- distance of COI sequences of *Tipula paludosa* and *T. oleracae* larvae for 2012 and 2013 data. Bootstrap support values (1000 replicates) are shown next to the branches; Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The analysis involved 150 nucleotide sequences. There were a total of 462 positions in the final dataset. All positions containing gaps and missing data were eliminated.

In order to further develop the sequencing method, adults of both species were included and aligned by ClustalW in MEGA 5.1 then the number of haplotypes (HN) was calculated using DnaSP v.5 (Librado & Rozas, 2009) for *T. paludosa* and *T*. *oleracea* adults and larvae. There were 18 haplotypes within *T. paludosa* larvae, and 10 within adults of the same species. For *T. oleracea*, 10 haplotypes were detected (4 for adults and 6 for larvae). A genetic P–distance table was constructed for each haplotype of larval *T. paludosa* and *oleracea*, and adult *T. paludosa* and *oleracea* (Table 3.1). All positions containing gaps and missing data were eliminated. There were a total of 469 positions in the final dataset. Bootstrap values of 1000 replicates were done to asses clade support. There was a high genetic P- distance between *T. paludosa* and *oleracea species* (Table 3.4) with an average of 6% P-distance indicating two different species within the same genus. There was a high genetic diversity within some *T*. *paludosa* haplotypes with a P-distance more than 3% of the standard metric of sequence divergence (Song *et al*., 2008). These samples include T49, T58, T63, L3, L34 and L64 (Table 3.4). Similarly, the within species P-distance within two larvae of *T*. *oleracea* (L37 and L103) was 4% thus, more than a standard metric sequence divergence (Table 3.4) and these are discussed next in section 5.4.

A Phylogenetic tree using the NJ method was constructed to visualise the relationship between *T*. *paludosa* and *T*. *oleracea* haplotypes (adults and larvae) in MEGA 5.1 using *T*. *recondita* as an outgroup (Figure 3.3). Both species occur in the tree as a separate clade with 78% bootstrap support between them. For *T. oleracea,* both L20 and L85 are closely related in the tree with 67% bootstrap support and these all occurred with other adult and larval *T*. *oleracea* as one

clade with 99% bootstrap support indicating a very strong relationship within these species collected in different fields. For *T*. *paludosa*, each of L34, T63, T49 were closely related with bootstrap 71% and L3 and T58 with bootstrap 90%. These two groups were very strongly related in one clade with 99% bootstrap support. L64 was placed in the tree as a monophyletic clade to other *T. paludosa* haplotypes with 76 % bootstrap support. T36 and L40 were closely related in the tree with 67% bootstrap support and placed as a basal node for L28, L33, T8 and L22 with 64% bootstrap supports whereas the haplotypes T64, L17 and L2 didn't match other haplotypes in the tree similar to T13, L55, T55, L4 and L166 (Figure 3.3). The samples T32 with L6, L105 with L126, L1 with T50 and L142 with L146 each were placed as a separate clade in tree with bootstrap supports 53%, 65%, 66% and 65% respectively and these were all closely related in a larger clade with 83% bootstrap support (Figure 3.3).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1-	T25 T. oleracea		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2-	T39 T. oleracea	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
3-	T40 T. oleracea	0.01	0.01		0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
4-	T41 T. oleracea	0.00	0.00	0.01		0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
5-	L19 T. oleracea	0.00	0.00	0.01	0.00		0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6-	L20 T. oleracea	0.00	0.01	0.01	0.01	0.00		0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
7-	L37 T. oleracea	0.01	0.01	0.01	0.01	0.01	0.01		0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
8-	L85 T. oleracea	0.00	0.00	0.01	0.00	0.00	0.00	0.01		0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
9-	L103 T. oleracea	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
10-	L104 T. oleracea	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.03		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
11-	T8 T. Paludosa	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.07	0.06		0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
12-	L1 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00		0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
13-	T13 T. Paludosa	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.08	0.06	0.00	0.00		0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
14-	T32 T. Paludosa	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.08	0.06	0.01	0.01	0.00		0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
15-	T36 T. Paludosa	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.00	0.00	0.00	0.01		0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
16-	T49 T. Paludosa	0.07	0.07	0.06	0.06	0.07	0.07	0.07	0.07	0.09	0.07	0.04	0.04	0.04	0.04	0.04		0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
17-	T50 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04		0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
18-	T55 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00		0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
19-	T58 T. Paludosa	0.06	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.08	0.07	0.03	0.04	0.04	0.04	0.03	0.01	0.04	0.04		0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
20-	T64 T. Paludosa	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.04		0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
21-	T63 T. Paludosa	0.06	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.08	0.07	0.03	0.04	0.04	0.04	0.03	0.00	0.04	0.04	0.00	0.04		0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
22-	L2 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.00	0.04	0.00		0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
23-	L3 T. Paludosa	0.06	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.08	0.07	0.03	0.04	0.04	0.04	0.03	0.01	0.04	0.04	0.00	0.04	0.00	0.04		0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
24-	L4 T. Paludosa	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.08	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04		0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
25-	L6 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00		0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
26-	L17 T. Paludosa	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.00		0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
27-	L22 T. Paludosa	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.00	0.00		0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
28-	L28 T. Paludosa	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.08	0.06	0.00	0.01	0.00	0.01	0.01	0.04	0.01	0.01	0.04	0.01	0.04	0.01	0.04	0.00	0.01	0.01	0.00		0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
29-	L33 T. Paludosa	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.08	0.06	0.00	0.01	0.00	0.01	0.01	0.04	0.01	0.01	0.04	0.01	0.04	0.01	0.04	0.00	0.01	0.01	0.00	0.00		0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
30-	L34 T. Paludosa	0.06	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.08	0.07	0.03	0.04	0.04	0.04	0.03	0.00	0.04	0.04	0.00	0.04	0.00	0.04	0.00	0.04	0.04	0.04	0.03	0.04	0.04		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
31-	L40 T. Paludosa	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.03		0.00	0.01	0.00	0.00	0.00	0.00	0.00
32-	L55 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.00		0.01	0.00	0.00	0.00	0.00	0.00
33-	L64 T. Paludosa	0.09	0.09	0.10	0.09	0.09	0.09	0.10	0.09	0.12	0.10	0.06	0.06	0.05	0.06	0.06	0.09	0.06	0.06	0.09	0.06	0.09	0.05	0.09	0.05	0.06	0.06	0.06	0.06	0.06	0.09	0.06	0.06		0.01	0.01	0.01	0.01	0.01
34-	L105 T. Paludosa	0.06	0.06	0.05	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.00	0.00	0.06		0.00	0.00	0.00	0.00
35-	L126 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.01	0.01	0.00	0.01	0.01	0.04	0.01	0.01	0.04	0.01	0.04	0.01	0.04	0.00	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.01	0.06	0.00		0.00	0.00	0.00
36-	L142 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.01	0.01	0.00	0.01	0.01	0.04	0.01	0.01	0.04	0.01	0.04	0.01	0.04	0.00	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.01	0.06	0.01	0.01		0.00	0.00
37-	L146 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.00	0.00	0.06	0.00	0.01	0.00		0.00
38-	L166 T. Paludosa	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.00	0.00	0.06	0.00	0.01	0.01	0.00	

Table 3.4 Pairwise P-distance of adult (T) and larval (L) *Tipula paludosa* and *T. oleracea* haplotypes. Standard errors are shown above the diagonal (blue) with 1000 bootsrap replicates. All gaps and mising data are eliminated.

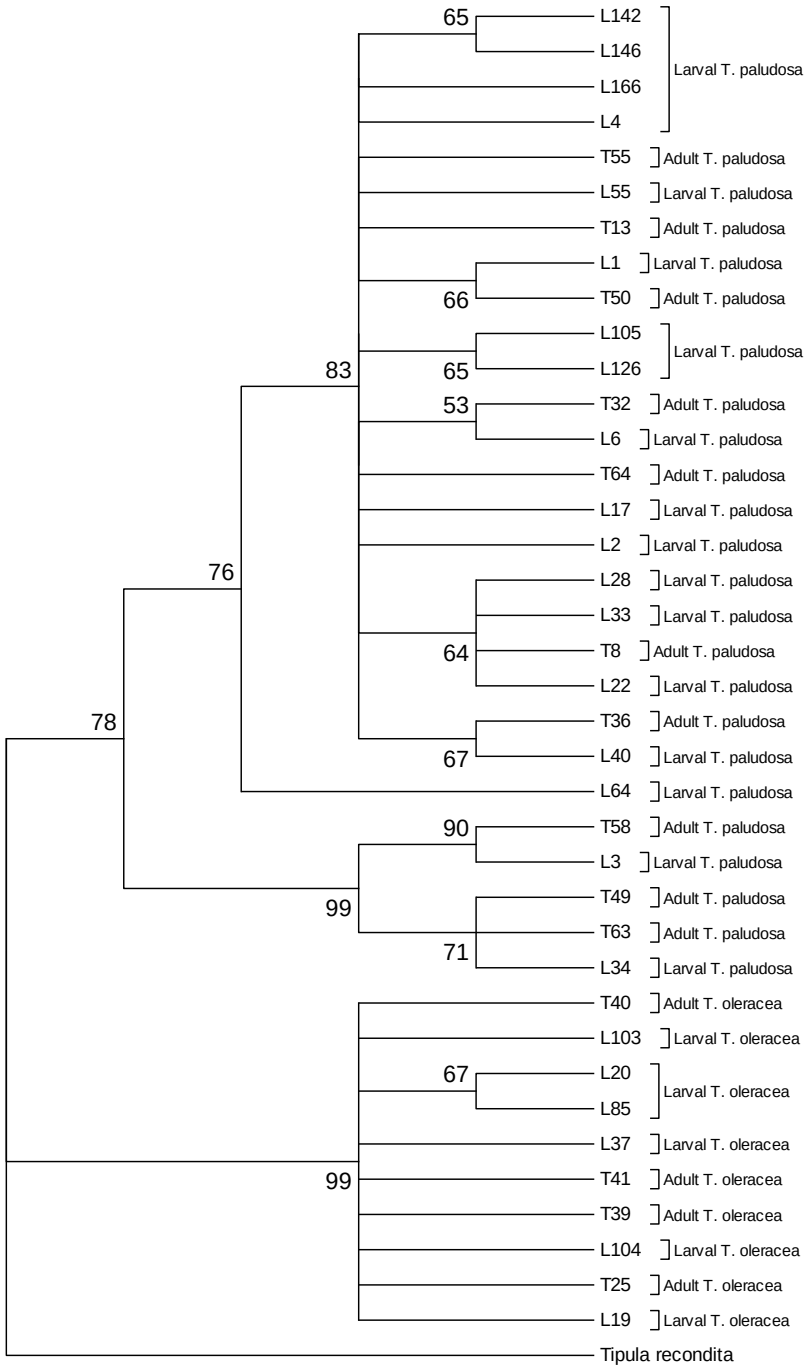


Figure 3.3 Neighbour Joining tree using p-distance of COI sequences of *T. paludosa* and *T. oleracae* (adults = T and larvae = L) haplotypes for 2012 and 2013 data. Bootstrap support values (1000 replicates) are shown next to the branches; Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The analysis involved 39 nucleotide sequences. There were a total of 462 positions in the final dataset. All positions containing gaps and missing data were eliminated.

3.4 Discussion

The species genetic identification showed that *T*. *paludosa* larvae are the most abundant species found in this study in comparison with *T*. *oleracea*. Among the larval samples sequenced, only 6 samples were found to be *T*. *oleracea* for the 2013 data out of about 100 larvae meaning that the proportion of the *T*. *oleracea* larvae is about 6% in comparison with *T*. *paludosa*. This is in agreement with a previous study in the UK by Humphreys *et al.*, (1993) who reported that *T*. *paludosa* is more prominent than *T*. *oleracea* in agricultural grassland, with the latter representing only 4% of larvae from a survey of 75 fields.

Phylogenetic analysis using NJ is the most popular method of constructing a phylogenetic tree of species data due to its speed and simplicity and MEGA is supposed to be the best program for estimating such tree (Tamur et al., 2011). The NJ tree shown here (Figure 3.1) represents the species genetic relationships within and between species of *T*. *paludosa* and *T*. *oleracea* larvae collected in 19 grass fields in 2012 and 2013. The tree has placed both species in a separate clade with 77% bootstrap support (Figure 3.1) and 0.06 genetic distance between them in average. Rao *et al.*, (2006) showed that the average genetic P-distance between the two species is 0.071 thus it is rather similar to the results obtained here indicating these two species as a sister group. There were within species genetic variations for both species of *T*. *paludosa* and *oleracea* that ranged from 0.1% to 3% generally; indicating that these specimens which were collected in both years and from each field are closely related to each other. In addition, the number of haplotypes for both species (adults and larvae) indicated that there are

genetic variations within both species collected from different fields (Table 3.4). Despite that, for some haplotypes (Table 3.4); there were high genetic variations within some species for both *T*. *paludosa* and *T*. *oleracea* haplotypes which was more than the standard metric sequence divergence (3%). For example, the genetic P-distance between (L37 and L103) was 4% for larval *T*. *oleracea*. Similarly, the genetic P-distance for the larval *T*. *paludosa* including T49, T58, T63, L3, L34 and L64 (Table 3.4) were more than 3% indicating that these may be misidentified due to the sequence quality and/or the presence of nuclear mitochondrial pseudogenes (numts). It is well recognised that the presence of numts could be problematic when amplifying DNA sequence data of the COI mitochondrial gene which can be co-amplified with the mitochondrial orthologue when using universal primers (Cui *et al*., 2013; den Tex *et al*., 2010; Moulton, Song & Whiting, 2010; Song *et al.*, 2008). The use of specific primers may help to reduce numts but this has not been thoroughly tested as some numts do not have stop codons or indels, thus discriminating these from mitochondrial orthologues is problematic (Moulton, Song & Whiting, 2010).

The other possibility of high genetic variability between these haplotypes particularly *T*. *paludosa* could refer to the possibility of other cryptic species close to *T*. *paludosa* as when these samples were identified from the BLAST database they only matched 96% identity to *T*. *paludosa* and similarly 96% to unknown *Tipula* species and this was noticed for some specimens of both larval and adult *T*. *paludosa.* Adults for which this was found were collected in random locations in the farm platform fields and nearby area; larvae, for example L149 with L160 and

separately L3 with L34, were found in the Ware Park and Long Lands South fields respectively. In addition, the other larvae such as L46, L64 and L97 were found in Top Burrows, Great and Long Lands North fields respectively and all these fields were located close to each other (Neighbour fields-see Figure 2.1) thus suggesting that this may be the preferred habitat for these highly diverged species of *T*. *paludosa* (Table 3.4), or that the limited dispersal noted for *T. paludosa* may result in this effect. The phylogenetic tree for the haplotypes (Figure 3.2) of *T*. *paludosa* showed that these larval samples are closely related to other highly diverged adult *T*. *paludosa*. For example, the haplotype L34 was placed in one clade in the tree with T49 and T63 (adult *T*. *paludosa*) with 71% bootstrap clade support and L3 with T58 with 90% bootstrap support. Both clades were placed together in the tree with a very strong relationship bootstrap support (99%) indicating that these are within the same species of highly diverged *T*. *paludosa* or even may be cryptic species close to them. Schowalter (2011) has reported that habitat fragmentation can isolate populations of a specific species and may limit their ability to maintain local populations making continual changes in community structure and ecosystem across landscapes, thus leading to maintain gene flow or diverge into separate species depending on the size and dispersal ability. The degree of gene exchange is influenced between demes depending on distances between demes as local demes are more influenced by genotypes of dispersants from neighbouring demes than by more distant demes (Schowalter, 2011). Habitat choices can also affect gene flow and these are made by dispersing individuals as when they enter a new area, they may prefer a specific habitat based on their phenotype or experience and this restrict their

interaction with other individuals that make different choices (Edelaar, Siepielski & Clobert, 2008). A further and more extensive soil sampling from these nearby fields (Ware Park, Long Lands South, Long Lands North, Top Burrows and Great fields) for larvae and adult sampling methods such as using light traps may provide a better understanding of these species and their genetic identification and diversity. Nevertheless, designing specific primers and comparing these with the universal primers used here may help a better understanding of these species identities.

In conclusion, the results presented here indicate that *T. paludosa* is the dominant species of leatherjacket found in grassland soils in comparison with *T. oleracea.* Additionally, the phylogenetic analysis presented here provides an initial view of the relationships between economically important leatherjackets of both species recovered here and within-species genetic variability for both species recovered at various fields and in different times of year. It also raises the question about the possibility of other cryptic leatherjackets in grassland soils which may together with *T. paludosa* and *T. oleracea* compose a species complex in grassland soils. Thus, future studies should address these questions through a more extensive sampling program for both adults and larvae and then identify them genetically. A phylogenetic relationship should then be obtained by comparing these and other species within the same genus to provide a better understanding species composition.

Chapter 4

General discussion and conclusions

Chapter 4: General discussion and conclusions

Over the last 100 years or so, there have been various studies investigating the abundance of soil insects in agricultural land (e.g. King, 1939; McAtee, 1907; Morris, 1920; Pantoja, Hagerty & Emmert, 2010b; Pollet, 2001; Rennie, 1917; Riggins, Davis & Hoback, 2009; Ross, Stapley & Cockbill, 1947; Turnock, Timlick & Palaniswamy, 1993) and some have focused on soil insects as complex agricultural pests (Benefer et al., 2012; Klein *et al.*, 2007). Additionally, there have also been various studies on the environmental factors responsible for the abundance and distribution of soil insects in agricultural land (Blackshaw & Hicks, 2012; Campos-Herrera *et al.*, 2006; Kabaluk *et al.*, 2005; Lepage *et al.*, 2012; Menta et al., 2011; Painter, 1936; Petersen, Seto & Peck, 2013; Wilkinson & Daugherty, 1970). However, there has been limited study on the abundance of soil insects in grassland systems and only a few have investigated the effect of sampling scale on the distribution of subterranean grassland insects (e.g. Benefer *et al.*, 2010) and none have investigated the farm scale effects on the distribution of the fauna in agricultural grassland. The recent developments in geographical information systems (GIS) for mapping and designing study areas and geographical positioning systems (GPS) for determining sampling points has helped researchers in biology and ecology to design new sampling methods for various ecological data. Here, in this study, a new 25 m sampling grid has been developed using GPS. Spatially accurate soil core sampling at each point of the grid led to an overall estimation of the population density of soil insects found in

agricultural grassland and understanding species distributions and their interactions at different sampling scales.

4.1 Sampling methods

Despite developing various sampling methods for estimating soil fauna populations (Blackshaw, 1994; Blasdale, 1974; Bynum & Archer, 1987; George, 1966; Parker & Howard, 2001; Ward & Keaster, 1977), the quality of the data could still be influenced if different sampling methods are applied for the same purpose; this can affect the quality of the results and hence the conclusions based on them (Barberena-Arias, González & Cuevas, 2012; Benefer *et al.*, 2012). Additionally, the sample size and accurate determination of the position of a sampling unit may considerably influence the development of spatial surveys (Rossi & Nuutinen, 2004).

Soil core sampling methods has been proven previously to be one of the most effective methods of sampling for many soil invertebrate species but each has used different sized soil cores depending on the area of study and the type of fauna (Blasdale, 1974; Mayor & Davies, 1976; Smith *et al.*, 1981). It has been proven previously to be faster, less laborious and/or more effective in comparison with other methods such as the ODCB method, bait traps and using saturated brine solution poured into 10cm plastic pipes pushed into pasture turf (Blackshaw, 1994; Parker & Howard, 2001; Stewart & Kozicki, 1987a). At low populations, the distribution of soil fauna may not be affected by sampling units but the influence may differ in the case of large populations and small sampling units may be more effective than larger ones (Finney, 1946). Here, we used a 6.5cm x 10cm soil

core sampling to estimate the population of the studied fauna and the modified heat extraction method based on Blasdale, (1974) to recover the fauna from soil samples.

The sampling scale influenced the spatial distribution of the taxa recovered in this study and also their interactions for both years of experiment. It is well recognised that scale is one of the most important factors affecting the spatial distribution of taxa, species diversity, interaction between species and relationship to environmental factors (Benefer *et al.*, 2012). The results in this study revealed that scale in a particular field as the most important factor in comparison with biotic and space variables influencing species distributions and their interactions in grassland systems. A recent study by Petersen, Seto and Peck (2013) showed spatial autocorrelation of *T*. *paludosa* larvae at the plot-level at lag distances ranging from 1.5 to 7 m and indicated that a 1m sampling distance is appropriate for the study of *T*. *paludosa* spatial distributions. This is, in particular, important for species of economic importance, such as leatherjackets in this study, when studying the effects of environmental and land use change on species distributions (Levin, 1992). The results in the current study also showed that the distributions of Tipulidae, Stratiomyidae, Carabidae (adults and larvae) and Muscidae changed in both years of study from random distribution to more aggregate at the field and farm scale, indicating the importance of scale on the distribution pattern of these taxa. Despite that, there were some taxa which were aggregated at all scales (Tables 2.1 and 2.4) but this altered according to their populations. For example, the family Chironomidae were found only in few numbers in comparison with Tipulidae in the second year survey and showed a

random distribution at all scales (Table 2.1) but the distribution changed to an aggregated pattern at all scales when populations were higher in the first survey year. At the same time, the degree of aggregation reduced from the farm scale to the core scale (Table2.4). Similarly, Benefer *et al.* (2010) showed that the distribution of wireworms and leatherjackets change from a random distribution at the core scale to a more aggregated distribution pattern at the site and field scales respectively.

4.2 Spatial Distribution

Knowledge about the distribution of soil insect pests in agricultural grassland is an important tool for population prediction and has implications in integrated pest management programs. Chapter 2 investigated the spatial distribution of soil insects in agricultural grassland at the field and farmlet scales. The spatial distribution of the taxa studied here plays an important role in the pest control programs of economic important pests such as leatherjackets in this study.

The results presented in chapter 2 for both years' data showed that leatherjackets are the dominant species of soil insects found this study in comparison with other taxa. Although low populations were found in both years when compared with previous reports (Blackshaw, 1984; Johnson & Murray, 2008), this is an important indication of population levels and in the case of patches of high population densities, will play a vital role in the control of such pests in specific areas of the field which are at risk. Therefore, if pesticides have to be applied, then this may lead to the reduction of pesticide applications.

There have been various studies on the factors that influence leatherjacket distributions such as: cultivation, particularly rolling and rotavation (Blackshaw, 1988), nitrogen content of the soil (McCracken, Foster & Kelly, 1995), silage (Purvis & Curry, 1981), natural enemies (see Blackshaw & Coll, (1999) for a review), rainfall (Mayor & Davies, 1976) and moisture (Coulson, 1962). Petersen, Seto and Peck (2013) suggested that the spatio-temporal distribution of leatherjackets is determined by habitat quality and predominantly influenced by soil moisture.

Surprisingly, despite being common in grasslands, only one wireworm was recovered in both years study when compared with other studies (Benefer *et al.*, 2010; Kleespies et al., 2013; Schallhart et al., 2011; Staudacher et al., 2013). This could be due to various factors such as soil type as wireworms are known to prefer heavy than light soils (Nadvornyj, 1968), soil structure (they prefer sand soils than silt and clay) (Ross, Stapley & Cockbill, 1948) and soil moisture (soils with high moisture are less suitable for wireworms) (Lefko et al., 1998). Falconer (1945) concluded that in England, wireworms are not normally liable to encounter lethal temperatures and suggested that the occurrence is not regular at temperatures above 30°C or below about -1°C. Lafrance (1968) found that wireworms moved deeper into the soil when soil temperature reached 19.4°C with only few numbers remained on the top layer of the soil and re-occurred again when temperature reached 22.2 °C at the end of August to mid-November when cold started again thus meaning that the seasonal distribution of wireworms are determined by changes in soil temperature (Fisher, Keaster & Fairchild, 1975).

Agriotes wireworms have also been found deeper in the soil during summer probably due to their response to high temperature and low moisture near soil surface (Parker, & Howard, 2001). They are known to be vulnerable to desiccation as their cuticle are permeable to water thus they lose water from their body at higher temperatures (Evans, 1944).

4.3 Genetic Identification and phylogeny of Tipulidae

The recent developments in new molecular methods such as DNA barcoding of the COI mitochondrial gene has become an important tool for the identification of many invertebrate species (Ashfaq *et al.*, 2013; Barr *et al.*, 2012; Boehme, Amendt & Zehner, 2012; Jung, Duwal & Lee, 2011; Lis, Lis & Ziaja, 2013; Park *et al.*, 2011b; Shin *et al.*, 2013). The universal primers (Vrijenhoek, 1994) tested here successfully amplified the target DNA of the COI mitochondrial gene of Tipulidae and was able to successfully differentiate the larvae of the two species. Although the adult samples were amplified successfully, the presence of PCR inhibitors in larval samples for both years' data caused a major problem in this study which prevented the amplification of target DNA during the PCR process. The use of BSA was an important step in overcoming PCR inhibitors and successfully amplification of target DNA of larval samples using universal primers suggesting that BSA is an amplification facilitator in DNA barcoding of larval Tipulidae. Juen and Traugott (2006) used BSA as an amplification facilitator to overcome PCR inhibitors in DNA-gut-content analysis of soil-living invertebrates and were able to overcome PCR inhibitors and detect prey (*Amphimallon solstitiale*, Coleoptera: Scarabaeidae) within the gut contents of predators

(*Poecilus versicolor* larvae, Coleoptera: Carabidae). BSA has also been used by other researches as an important PCR amplification facilitator (Al-Soud & Rådström, 2000; Al-Soud & Rådström, 2001; Liang & Keeley, 2011; Wang, Olson & Chang, 2007) through binding and neutralizing PCR inhibitors (Repetto et al., 2013).

The identifications for both years' Tipulidae larvae showed that the majority of the samples (94%) are *T. paludosa* suggesting that *T. paludosa* is the most abundant of the two species recovered in this agricultural grassland habitat. These results confirm the findings of Humphreys *et al.* (1993) that *T. oleracea* are not found frequently in UK agricultural grassland, though recently *T. oleracea* have become common in turfgrass of the Northeast United States (Peck & Olmstead, 2009) thus their differentiation is important in terms of pest management programs (Rao *et al.*, 2006).

In summary, the objectives of this research program was to estimate populations of soil insects found in agricultural grassland at three different spatial scales (Core, field and farmlet), the effect of sampling scale on species distributions and their interactions, and to identify and estimate proportion of *T. paludosa* to those of *oleracea* larvae in grassland. Hence, a number of methodological conclusions and research perspectives can be drawn from this research program:

Firstly, using a systematic sampling program for both years survey, our results has shown that the populations of soil insects are generally low in the Farm Platform in south Devon at the core, field and farmlet scales in comparison with

other reports from the UK and that *T. paludosa* is the dominant species of all taxa recovered. These findings confirm other reports in the UK who demonstrated leatherjackets as one of the dominant grassland insects (Benefer, C. M., 2012 and Smith, 1989). Secondly, it also confirms previous reports (e.g. Benefer *et al,* 2010) that argue that scale is an important factor affecting the distribution of soil insects in grassland. The Scale effect is an important issue and can be found in almost all environmental applications. A particular attention should be paid to scale issues when investigating spatial distribution of soil fauna. The major questions are related to the identification of appropriate scales for the representation, observation and analysis and also to implementation of scale analysis that can improve our understanding of species distribution within a particular scale.

Results from this case study provide guidelines to enrich the existing Information on the spatial distribution of soil insects in agricultural grassland through the sampling program and the three spatial scales investigated and the molecular techniques used to identify leatherjackets. Therefore, future research should focus on the development of specific sampling programs for the individual taxa and to develop a model that would better address species distribution within a particular scale. The molecular methods such as the DNA sequencing could help as an important tool for the identification of other soil taxa to species level. For example, the universal DNA primers used here may be applied to other taxa and give a better understanding of species specific distribution. Finally, relating the distribution of taxa to environmental factors such as temperature and moisture

may help a better understanding species distribution and the factors affecting them within a particular scale and this could play a principal role in the integrated pest management of economically important species in agricultural grassland.

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Appendices:

Appendix 1

a- A *Tipula paludosa* larva - Posterior spiracle.

b- Adult *Tipula paludosa* - Male genitalia.

c- A Bibionidae larva

d- A Dolichopodidae larva- (Type B)

e- A Dolichopodidae larva- (Type B)-Posterior spiracle.

f- Psychodidae larvae

g- An adult Carabidae

h- A Carabidae Larva

i- A Click beetle Larva (Wireworm)

j- A Cantharidae larva

k- Chrysomelidae larvae

l- A Noctuidae larva

m- A Chironomidae larva

n- Sciaridae larvae

o- A Stratiomyidae larva

Appendix 2 Gel electrophoresis – to check DNA quality

To make a 2% gel, add 100ml buffer (1x TBE) and 10µl SYBR gel stain to a conical flask, then add 2g agarose and gently mix. After that heat in the microwave for approx. 2 minutes until the liquid is bubbling (be careful that it doesn't overflow) and all agarose has been dissolved (it should be clear). Leave to stand until hand-hot. While the gel is cooling, prepare a gel casting tray. Place up to two combs (depending on how many samples there are) into the slots on the tray, and tape the ends securely to prevent the gel leaking. Place on a flat surface. When the gel is cool enough to hold, slowly pour into the tray. Remove any bubbles with a clean pipette tip and leave to cool until solid (and it has turned slightly opaque) then remove the combs and place the tray into a gel tank (which contains 1X TBE buffer). Add 3µl of loading buffer to 5µl of the DNA extract for each sample. Add 5µl of ladder to the first well of the gel. In each of the next wells, add all of the sample/loading buffer mixture for each sample. Make a note of which samples were added to which wells. Place the lid of the gel electrophoresis equipment on and attach wires to the corresponding points on the control unit. Set the running time to 1-1.5 hours and the voltage to 110V (you may have to change the mA setting to get the correct voltage) and press start. When it has finished, remove the tray from the equipment. Carefully place the gel into the gel imaging system to reveal whether the extraction has worked.

Appendix 3

Examples of haplotypes Identified in BOLD and BLAST data bases

Sample T25 **Identification** *T. oleracea* **%Match** 100

ATGCATTTATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAG
TTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTT
GATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCG
GAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGACC
CTG

Sample T39 **Identification** *T. oleracea* **%Match** 99.5

ATGCATTTATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCTCTTACTCTTTTATTAGCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAG
TTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTT
GATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCG
GAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGACC
CTG

Sample T40 **Identification** *T. oleracea* **%Match** 99.51

ATGCATTTATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGGGCCCCAGATATGGCTTTTCCACGA
ATAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCT
AGTAGTATAGTGGAAAATGGGGCTGGAACAGGTTGAACCGTTTACCCCCCA
CTTTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTC
TCTTCATTTAGCAGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTAC
AGTTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGT

TTGATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGC
CGGAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTG

ACCCTG

Sample T41 **Identification** *T. oleracea* **%Match** 99.84

ATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTCCCACGA
ATAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCT
AGTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCA
CTTTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTC
TCTTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTAC
AGTTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGT
TTGATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGC
CGGAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGA
CCCTG

Sample L19 **Identification** *T. oleracea* **%Match** 100

ATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAG
TTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTT
GATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCG
GAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGACC
CTG

Sample L20 **Identification** *T. oleracea* **%Match** 99.52

ATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCTCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAG

TTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTT
GATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCG
GAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGACC
CCG

Sample L37 **Identification** *T. oleracea* **%Match** 99.06

ATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCAGTTAGCGGGAATTTCCTCGATTTTAGGAGCTGTAAATTTTATTACTACA
GTTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTT
TGATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTGTACCTGTTTTAGCC
GGAGCTATGAAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGAC
CCTG

Sample L85 **Identification** *T. oleracea* **%Match** 99.83

ATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCTCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAG
TTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTT
GATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCG
GAGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGACC
CTG

Sample L103 **Identification** *T. oleracea* **%Match** 94

ATGAATTTATTATAATTTTTTTTATAGTTATAAATATTATAATTGGAGGATTTGG
AAATTGATTAGTTTTTTTAATAGTAGGATGTTGAGATATGGTTTTTCCACGAAT
AAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATTAGCTAG
TAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCACTT
TCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTCT

TCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAGT
TATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTTG
ATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCGG
AGCTATTACAATATTATTAACAGACCGAAATTTAAATACCTCTTTTTTTGACCC
TG

Sample L104 **Identification** *T. oleracea* **%Match:** 99.68

ATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTCCCTTTAATACTAGGAGCCCCAGATATGGCTTTTCCACGAA
TAAATAATATGAGTTTTTGGATATTACCCCCATCCCTTACTCTTTTATAACCTA
GTAGTATAGTGGAAAATGGAGCTGGAACAGGTTGAACCGTTTACCCCCCACT
TTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGATCTAGCTATTTTTTCTC
TTCATTTAGCGGGAATTTCCTCTATTTTAGGAGCTGTAAATTTTATTACTACAG
TTATTAATATACGATCAAGAGGAATTACATTAGATCGAATACCATTATTTGTTT
GATCAGTAGTGATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCCG
GAGCTATTACAATATTATTAACAGACCGAAATTAAATACCTCTTTTTTTGACCC
TG

Sample T8 **Identification** *T. paludosa* **%Match** 99.83

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTAGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L1 **Identification** *T. paludosa* **%Match** 99.53

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGGGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC

ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample T13 **Identification** *T. paludosa* **%Match** 100

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample T32 **Identification** *T. paludosa* **%Match** 99.83

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATACTAGGAGCCCCAGATATAGCTTTTCCCCGA
ATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGCT
AGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACCA
CTTTCTGCCAGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTTTC
TCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTA
GCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GACCCTG

Sample T36 **Identification** *T. paludosa* **%Match** 99.83

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC

TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample T49 **Identification** *T. paludosa* **%Match** 96.08

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGGTTTG
GAAATTGATTAGTTCCTTTAATGCTAGGGGCTCCAGATATAGCTTTCCCCCG
AATAAATAATATAAGTTTTTGAATATTACCTCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTCTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGACCTAGCTATTTTTT
CTCTTCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAATTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCCGTTTTATTACTTCTATCTTTACCTGTTCTA
GCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GATCCTG

Sample T50 **Identification** *T. paludosa* **%Match** 99.83

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGGGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample T55 **Identification** *T. paludosa* **%Match** 99.7

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATCGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG

AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample T58 **Identification** *T. paludosa* %Match 96.57

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGGTTTG
GAAATTGATTAGTTCCTTTAATGCTAGGGGCTCCAGATATAGCTTTCCCCCG
AATAAATAATATAAGTTTTTGAATATTACCTCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTCTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGACCTAGCTATTTTTT
CTCTTCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTTTATCTTTACCTGTTCTA
GCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GATCCTG

Sample T64 **Identification** *T. paludosa* **%Match** 100

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATACTAGGAGCCCCAGATATAGCTTTTCCCCGA
ATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGCT
AGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACCA
CTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample T63 **Identification** *T. paludosa* %Match 96.42

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGGTTTG
GAAATTGATTAGTTCCTTTAATGCTAGGGGCTCCAGATATAGCTTTCCCCCG
AATAAATAATATAAGTTTTTGAATATTACCTCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTCTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGACCTAGCTATTTTTT
CTCTTCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCCGTTTTATTACTTCTATCTTTACCTGTTCTA
GCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GATCCTG

Sample L2 **Identification** *T. paludosa* **%Match** 99.84

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCGTCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample L3 **Identification** *T. paludosa* **%Match** 96.0

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGGTTTG
GAAATTGATTAGTTCCTTTAATGCTAGGGGCTCCAGATATAGCTTTCCCCCG
AATAAATAATATAAGTTTTTGAATATTACCTCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTCTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGACCTAGCTATTTTTT
CTCTTCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTTTATCTTTACCTGTTCTA
GCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GATCCTG

Sample L4 **Identification** *T. paludosa* **%Match** 100

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample L6 **Identification** *T. paludosa* **%Match** 99.84

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCAGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L17 **Identification** *T. paludosa* **%Match** 100

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATACTAGGAGCCCCAGATATAGCTTTTCCCCGA
ATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGCT
AGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACCA
CTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L22 **Identification** *T. paludosa* **%Match** 99.84

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTAGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L28 **Identification** *T. paludosa* **%Match** 99.52

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCATTTACCCACCA
CTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTAGCTATTTTTTC
TCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTA
GCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GACCCTG

Sample L33 **Identification** *T. paludosa* **%Match** 99.68

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTAGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATCACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L34 **Identification** *T. paludosa* **%Match** 96.0

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGGTTTG
GAAATTGATTAGTTCCTTTAATGCTAGGGGCTCCAGATATAGCTTTCCCCCG
AATAAATAATATAAGTTTTTGAATATTACCTCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTCTCTGCTGGAATTGCTCATACAGGAGCATCAGTAGACCTAGCTATTTTTT
CTCTTCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACTA
CAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATTT
GTCTGATCAGTAGTAATTACTGCCGTTTTATTACTTCTATCTTTACCTGTTCTA
GCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTT
GATCCTG

Sample L40 **Identification** *T. paludosa* **%Match** 99.84

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample L55 **Identification** *T. paludosa* **%Match** 99.84

ATGCATTTATTATAATTTTTTTTATAGATATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTAT
TTGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTT
TAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTT
TTGACCCTG

Sample L64 **Identification** *T. paludosa* %Match 95

ATGCTTTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAATTCCTTTAATGCTGGGAGCCCCAGATATAGCCTTTCCCCG
AATAAACAATATAAGATTTTGAATATTACCCCCTTCCCTTACCCTTTTGTTAGC
TAGAAGAATAGTGGAAAACGGAGCTGGAACAGGATGAACCGTTTACCCTCC
TCTTTCTGCCGGAGTTGCCCATACAGGAGCTTCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCACAGGAATTACATTTGACCGAATCCATTATTT
GTTGATCGTAGAATTACTGCTGTATTATTACTTCTTTCTTTACCTGTTTTACTG
GGCTATTACAATATTATTAACTGACCGAATTTTATACCTCTTTTTTTGACCTG

Sample L105 **Identification** *T. paludosa* **%Match** 99.35

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCAGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L126 **Identification** *T. paludosa* %Match 97

TTGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCAGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L142 **Identification** *T. paludosa* **%Match** 99.16

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAAATATAGCTTTTCCCCGA
ATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGCT
AGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACCA
CTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTTTCTAGGAGCTGAAATTTTTTACTACA
GTTATTAATATACGATCAAGAGGAATTATTTAGACCGAATCCATTATTTGTCT
GATCAGAGAAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTTAGCTGG
GGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTTTGACCC
TG

Sample L146 **Identification** *T. paludosa* **%Match** 99.67

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAAATATAGCTTTTCCCCGA
ATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGCT
AGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACCA
CTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTTT
CTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTACT
ACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATACCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Sample L166 **Identification** *T. paludosa* **%Match** 99.83

ATGCATTTATTATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGATTTG
GAAACTGATTAGTTCCTTTAATGCTAGGAGCCCCAGATATAGCTTTTCCCCG
AATAAATAATATAAGTTTTTGAATATTACCCCCATCCCTTACCCTTTTATTAGC
TAGTAGTATAGTGGAAAACGGGGCTGGAACAGGTTGAACCGTTTACCCACC
ACTTTCTGCCGGAATTGCCCATACAGGAGCATCAGTAGATCTGGCTATTTTT
TCTCTCCACTTAGCGGGAATTTCTTCTATTCTAGGAGCTGTAAATTTTATTAC
TACAGTTATTAATATACGATCAAGAGGAATTACATTAGACCGAATATCATTATT
TGTCTGATCAGTAGTAATTACTGCTGTTTTATTACTTCTTTCTTTACCTGTTTT
AGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCTCTTTTTT
TGACCCTG

Appendix 4

RESEARCH WITH PLYMOUTH UNIVERSITY

Systematic sampling of agricultural grassland to investigate the spatial distribution of soil insects

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ROTHAMSTED RESEARCH

Introduction

Grasslands comprise the primary vegetation in many environments. Natural grasslands cover millions of hectares across the world, supporting a wide range of wild life (Klein et al., 2007).

In the UK, grasslands comprise a predominant part of the agricultural land in the country and are the basis of livestock production (FAO, 2008).

Grassland and other agricultural land	England	wales	Scotland	N.Ireland	All UK
Crops and tillage	3 840	66	1 566	187	5 659
Grass < 5 years old	590	115	325	136	1 166
Grass > 5 years old	3 330	982	910	676	5 898
Rough grazing in sole rights	640	221	3 340	149	4 350
Common rough grazing	395	180	598	29	1 202
Total agricultural land†	8 795	1 564	6 739	1 177	18 275
†excludes woodland on farms and set-aside land					

In the UK, grasslands also provide a habitata for several subterranean insect taxa some of which are pests - leatherjackets (larvae of Tipulidae: Diptera) and wire worms (larvae of Elateridae:Coleoptera) (Benefer, C. M. 2011). To provide an appropriate method of pest management, a systematic sampling of agricultural grassland is crucial in order to understand their population distribution and distribution patterns within a farm. Hence, the aim of the present study was to collect data on their spatial distribution and distribution patterns in agricultural grassland.

Methods

Study site and soil sampling

The study was conducted at the Rothamsted, North Wyke Farm Platform near Oakhampton, Devon, UK. (Fig.1). Soil samples were taken from 19 permanent grass fields at 25m spacing. (1131 soil cores from 67.2 ha).

Samples were taken using a 6.5 cm diameter soil core with depth of 15 cm and put in plastic bags with labels for laboratory processing.

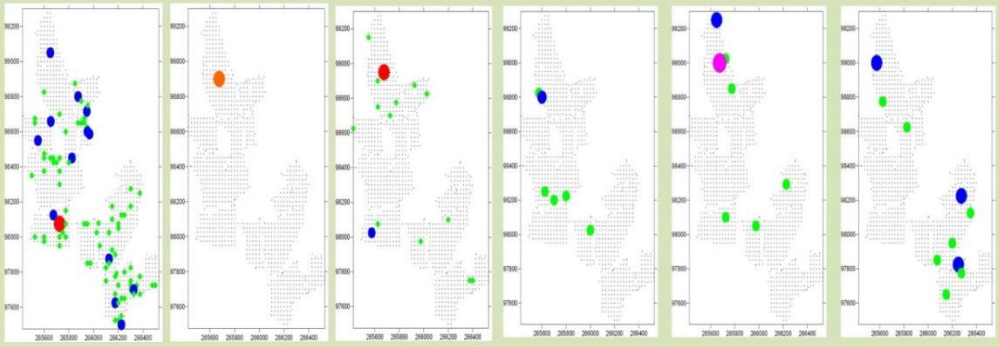
Figure 1: North Wyke Farm plat form

Extraction and Identification of taxa

Insects were extracted using a modified Blasdale heat system (Fig. 2). A total of 233 insects were recovered and identified primarily into to family level which were larvae of Tipulidae, Sciaridae, Stratiomyidae, Muscidae, Carabidae (adult and larvae). A further nine larvae await identification. larvae. Distribution maps are shown for the named taxa (Fig. 3).

Figure 2: Heat Extraction Method

Results



Tipulidae | Sciaridae | Stratiomyidae | Muscidae | Carabidae-adults | Carabidae - larvae

Figure 3: Distribution maps of different subterranean insect taxa in grassland - North Wyke Farm Plat form. The count scales are green = 1, blue = 2 and red = 3+ per core.

Future work

- Develop a molecular method to differentiate between T. oleracea and T. paludosa larvae
- Analyse the spatial patterns
- Relate the insect distributions to each other and environmental variables

References

Benefer, C. M. (2011) 'The Molecular and behavioural ecology of click beetles (Coleoptera: Elateridae) in agricultural land', .PhD thesis .University of Plymouth.

Blasdale, P. (1974) 'A method of turf sampling and extraction of leatherjackets', Plant Pathology, 23 (1), pp. 14-16.

Buckle, P. (1923) 'On the ecology of soil insects on agricultural land', Journal of Ecology, 11 (1), pp. 93-102.

FAO, (2008) ' country pasture /forage resource profile, united kingdom', available at: http://www.fao.org/ag/AGP/AGPC/doc/Counprof/britain/unitedkingdom.htm,

Klein, M. G., Grewal, P. S., Jackson, T. A. & Koppenhöfer, A. M. (2007) 'Lawn, turf and grassland pests', Field manual of techniques in invertebrate pathology, pp. 655-675.

Appendix 5

Presentations and conferences attended

1- Soil ecology and entomology annual meeting, National Marine Aquarium, Plymouth UK, 14/9/2011 to 16/9/2011.

2- 'Systematic sampling of agricultural grassland to investigate the spatial distribution of soil insects'. Ento'12 Royal Entomological Society annual meeting, Cambridge - UK, 18-20 / 7 / 2012.

Workshops and taught sessions

1- Geographical Information Systems (GIS): an introduction- Plymouth University, UK, 8 November 2011.

2- Advanced Spider identification work shop, Plymouth University, UK, 20 April 2013.

3- GLIM, GLAM & GLUM Statistics Course, Plymouth University, UK,16-17 April and 4-5 June 2013.

Taught modules

1- BIOL3209 -Crop production and protection - September 2011 to march 2012

2- Postgraduate research skills and methods- October 2011 to December 2011

3-Laboratory based teaching methods and practice -October 2011 to December 2011

4- Principles of electron microscopy - October 2011 to December 2011