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The effect of glyphosate and nitrogen on plant communities and the soil fauna in terrestrial biotopes at field margins

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Foreword

On November 1, 2011, The Danish Ministry of the Environment notified the Department of Bioscience, Aarhus University, that they committed to granting the project: The effect of glyphosate and nitrogen on plant communities and the soil fauna in terrestrial biotopes at field margins, with Professor Christian Damgaard, Department of Bioscience, Aarhus University, as the project manager. This report is a compilation of the results of the results and conclusions of the project.

Selected results will also be published in international peer-reviewed journals.

Summary

The aim of the study was to improve our understanding of and be able to quantify and predict the effects of glyphosate and nitrogen and their interaction on small terrestrial biotopes in the agricultural landscape, e.g. hedgerows and field margins. For both vegetation and soil fauna, the effects were assessed at the ecosystem level by measuring biodiversity and functional traits.

We have obtained an increased understanding of the causal relationship between plant communities and the soil fauna at the ecosystem level and increased knowledge on how and by what mechanisms important drivers that are known to affect plant communities may affect pollination and the soil fauna. The combined use of plant trait and soil fauna trait data in a full-factorial field experiment of glyphosate and nitrogen has never been explored before. The focus on plant and soil fauna traits rather than species enabled a robust description of the ecological processes at the functional level.

More specifically, both fertilizers and herbicides affected species composition. Generally, species cover decreased with increasing glyphosate doses, although cover of *Festuca ovina* and *Euphorbia esula* forms exceptions. Increasing nitrogen, generally, resulted in increasing total plant cover and biomass, especially of fast-growing and competitive species as grasses and a few herbs such as *Tanacetum vulgare*.

Using plant traits we found that increase in nitrogen promoted an increase in the average specific leaf area (SLA) and canopy height, whereas glyphosate promoted a decrease in those traits. Additionally, the present analysis found an increase in competitive ability (increase in Ellenberg N and Grimes C and a decrease in Grimes S) with increasing nitrogen. With increasing doses of glyphosate, the present analysis also found an increase in Grimes S. For the two composite species *Tanacetum vulgare* and *Leucanthemum vulgare*, the two most heavily affected traits were floral density and flowering phenology, in turn leading to marked changes in plant-pollinator interactions.

Nitrogen application caused a shift towards earthworms feeding on litter. There was a negative relationship between glyphosate and habitat width of Collembola, whereas nitrogen had the opposite effect. Deep root biomass was positively correlated with trophic position of Collembola. We observed both stimulation and declines of microarthropod populations in response to the high N fertilization.

The empirical data of vegetation and soil fauna biodiversity and traits was linked to the underlying ecological processes at the functional level of the ecosystem using the modelling approach of structural equation modelling.

Sammenfatning

Formålet med denne undersøgelse var at øge forståelsen af og kvantificere og forudsige effekten af glyfosat og kvælstof og deres samspil på seminaturlige småbiotper i landbrugslandskabet, fx levende hegn og markskel. For både vegetationen og for jordbundsfaunaen blev effekterne vurderet på økosystemsniveau ved at måle biodiversitet og funktionelle egenskaber.

Vi fik et forøget kendskab til årsagssammenhængen mellem plantesamfund og jordbundsfaunaen på økosystemsniveau og bedre viden om, hvordan, og ved hjælp af hvilke mekanismer, vigtige påvirkningsfaktorer påvirker plantesamfund, bestøvning og jordbundsfaunaen. Der har ikke tidligere været forsøg med en kombineret brug af egenskabsdata for både planter og jordbundsfauna i et realistisk feltforsøg med glyfosat og kvælstof. Ved at fokusere på egenskaber i stedet for på arter var det muligt at lave en grundig beskrivelse af de økologiske processer.
Helt konkret påvirkede både kunstgødning og herbicider artssammensætningen. Generelt faldt plantedækningen med stigende doser af glyfosat, med undtagelse af dækningen af *Festuca ovina* og *Euphorbia esula*. Stigende kvælstof resulterede generelt i en stigning i plantedækningen og biomasse, især for hurtigt voksende og konkurrencedygtige arter som græsser og enkelte urter såsom *Tanacetum vulgare*.

Ved at bruge planteegenskaber fandt vi, at øget kvælstof medførte en stigning i det gennemsnitlige specifikke bladareal og plantens højde, hvorimod glyfosat medførte en nedgang af disse egenskaber. Yderligere viste analyserne en stigning i Ellenberg N og Grimes C og Grimes S i forbindelse med stigende kvælstof. Undersøgelsen viste også, at Grimes S steg med stigenden glyfosat doser. For de to kurvblomstrede planter *Tanacetum vulgare* og *Leucanthemum vulgare* var de to mest påvirkede egenskaber tætheden af blomster og blomstringsfænologi, hvilke kan lede til markante forandringer i plante-bestøver interaktioner.

Der var en negativ sammenhæng mellem glyfosat og habitatsbredden for Collembola, hvorimod kvælstof havde den modsatte effekt. Biomassen for dybe rødder var positivt korreleret med den trofiske niveau af Collembola. Vi observerede både stimulation og nedgang i populationer af mikro-leddyr som følge af stigende kvælstof.

De empiriske data for vegetations- og jordbundsfauna biodiversitet og egenskaber blev knyttet til de underliggende økologiske processer ved hjælp af strukturelle lignings modeller.

1. Introduction

The biodiversity of most living organisms within European agricultural areas is declining (Green 1990, Fuller et al. 1995, Rich and Woodruff 1996, Chamberlain et al. 2000, Donald et al. 2000, Benton et al. 2002, although see also Andreasen and Stryhn 2008). A number of factors, often summarized as the intensification of the agricultural practice and loss of habitats, are made responsible for the decline. Specifically, the repeated application of fertilizers and pesticide usage are generally regarded to play important roles for the decline.

There is increasing knowledge of the effects of applying either fertilizers or herbicides to the biodiversity of higher plants in the agro-ecosystem. However, studies of the combined effect of fertilizers and herbicide drift on non-target vegetation are scarce (see references in Strandberg et al. 2012), even though the two important drivers often co-occur in small terrestrial biotopes at field margins. As a response to this important knowledge gap, a replicated long-time field experiment with the herbicides glyphosate and nitrogen was set-up (Bruus Pedersen et al. 2004, Holst et al. 2008, Strandberg et al. 2012). This experiment has now been running for ten years and is the *only* field experimental site, worldwide, where it is possible to test hypotheses on the combined effect of glyphosate and nitrogen fertilisation on biodiversity and ecological properties and function in terrestrial biotopes at field margins in a realistic way at the ecosystem level. The only comparable experiment that we know of is a field study near Landau, Germany, where the herbicide sulfonylurea is investigated in combination with the insecticide pyrethroid, and nitrogen (Schmidt et al. 2013).

Environmental drivers, such as the use of pesticides and nitrogen, may lead to selection of more adapted plant phenotypes in plant communities (Garnier et al. 2007), but since different plant species share a similar contingent of plant traits, a more or less deterministic selection process at the plant trait level does not necessarily lead to the invasion of the same species in different sites (Shipley 2010). Instead, the change (and the rate of change) in species composition will vary among sites and depend on the existing plant community, the content in the seed bank, and the possibility of new plant species invading from neighbouring semi-natural habitats in the agricultural landscape (Milchunas and Lauenroth 1995). However, the plant traits of the different species are characteristic features of the survival, growth and reproductive strategies of the particular species and are, thus, expected to respond in a more predictable way to an altered environment than the observed change in species composition (Shipley et al. 2006, Vile et al. 2006, Garnier et al. 2007, Shipley 2010). Consequently, in order to be able to characterize the selection responses of both the vegetation and the soil fauna at various treatments of glyphosate and nitrogen, it was decided to focus on the change in abundance of different relevant functional traits rather than examining the change in the abundance of the different species. Furthermore, it has been shown that it is useful to consider ecosystem service providers in terms of their traits rather than their taxonomy (Anton et al. 2010).

Soil fauna traits have a significant explanatory power when understanding pesticide effects, climatic effects and general habitat preferences and responses (Faber 1991, Krogh 1991, de Bello et al. 2010, Krab et al. 2010, Vandewalle et al. 2010, Makkonen et al. 2011). Furthermore, within soil ecology it has become a truism that there is no general link between soil fauna biodiversity and single plant species. Consequently, the intimate link between plants and soil organisms is certainly based on general plant traits related to their creation of habitat and food sources. Here, food sources would

be both direct through root herbivory and indirect through feeding on microbial decomposers feeding on the root and root exudates.

In order to facilitate the translation of ecological information into future economic models, incentives and governance (Daily and Matson 2008), there is an urgent need to provide the necessary ecological information. A key element of the ecosystem services paradigm is the ecosystem properties and functions, which are the direct drivers of the ecosystem services. Here, we make a first attempt to describe the biodiversity underlying the agro-ecosystem functions during the impact of pesticides and inorganic fertilisers in terms of traits.

Pollination is a crucial ecosystem function essential for maintenance of crop quantity and quality, and insect pollinators play a key role for all insect-pollinated plants, including both crops and wild species. Globally, about 87.5 % of the flowering plants are animal pollinated (Ollerton 2011). The proportion depends on the climate zone. Within the temperate zone, the proportion is 78 %, and more than 80 % of European crops depend, at least in part, on insect pollination. In Denmark, 75 % of the crops are fully or partially insect pollinated, and the pollination services for Danish crops is estimated to be worth 646 million DKK per year (Axelsen 2011 (Available 1. May)). Application of both fertilizers and pesticides has the potential to affect pollination. Fertilization may result both in changes in species composition (Holst 2008, Strandberg 2011 (in press)) and in larger flowers and, consequently, in more pollen and nectar (Petanidou 1999, Gardener 2001, Spaethe 2001, Thompson 2001, Cartar 2004). Herbicides, on the other hand, may result in reduced flowering (Strandberg submitted) and changes of both quantity and quality of pollen and nectar (Kearns 1998, Petanidou 1999).

The soil ecological functions relying on the interplay between plants and soil fauna are the well-known cycling of plant nutrients, regulation of fungi, maintenance of soil structure and water retention (Brussaard et al. 2007). Many of the functions performed by soil organisms that contribute to ecosystem services are supporting services, which indirectly underpin the provisioning of all other ecosystem services. Because soil fauna interacts with soil microorganisms, which we do not cover in this project, their role becomes rather broad, so five groups of functions can be identified involving soil fauna: i) soil structure, soil organic matter and fertility; ii) regulation of carbon flux and climate control; iii) regulation of the water cycle; iv) decontamination and bioremediation; and v) pest control (Turbé et al. 2010).

The soil food web structure can be revealed by means of a quantitative analysis of stable isotopes. This approach has been used now for two to three decades in soil ecology and provides an overview of the trophic structure reflecting a basic and cohesive element of the soil ecosystem. Obvioulsy, a comprehensive coverage of this complex and diverse system depends on the extent that the constituent system elements have been collected and included in the analysis (Spain et al. 1990, Briones et al. 1999a, Traugott et al. 2013). Stable isotopes have not yet been used in studies of changes in soil-ecosystems under the influence of external potential stressors such as pesticides and fertilizers. Exeptions is Girard et al. (2011) who studied the relationship between fertilization and crops, and Birkhofer et al. (2011) that studied generalist predators in organic and conventional farming systems. There are some rules of thumb that suggest that $\delta^{15}N$ increases by 3 ‰, written as $\Delta^{15}N = 3$ ‰, from one trophic level to the next, and $\delta^{13}C$ with 1 ‰. These changes or *trophic shifts* are highly variable, and there is still a need to define them more generally (Perkins et al. 2014). However, in the present context where we aim to detect changes in the trophic structure of basically identically ecosystems due to the imposed impact of glyphosate and N ferilization, the exact size of these trophic shifts is therefore not critical. Our analysis consist of selecting the individual species and test statistically if their position in the trophic structure, also termed their isotopic niche {Korobushkin, 2014 #5461}, is affected by the treatment factors. The general hypothesis, whether the experimental treatments create changes in the trophic position, is supported by the experimental design of the field experiment.

Recently, there has been a remarkable advance in the empirical modelling of ecological processes using SEM that allows parameterizing complex models with many parameters and latent variables, and it has been demonstrated how the use of these modelling techniques provides unprecedented possibilities for detecting causal ecological relationships, testing compound ecological hypotheses, and making ecological predictions with quantitative estimates of the uncertainty that is associated with ecological predictions (e.g., Grace et al. 2010, Damgaard et al. 2014).

Important information on the effect of glyphosate and nitrogen and their interactions on the abundance and competitive interactions of plant species has been collected at the experimental site "Kaløplottet" (e.g. Bruus Pedersen et al. 2004, Holst et al. 2008, Damgaard et al. 2011, Strandberg et al. 2012, Damgaard et al. 2013), but there is no information on the effects on the soil fauna or the interaction between the effects on the vegetation and the soil fauna at the ecosystem level. However, since glyphosate has no likely direct effects on soil fauna at the recommended field dose, indirect cascading effects through the composition of the plant community are expected to the most important effect of glyphosate on soil fauna biodiversity.

Very little is known about the combined effects of fertilizers and herbicides on pollination. The Kalø experimental plot offers the potential to look for such effects on pollination, and in the STEP-project (Status and Trends of European Pollinators) we study effects of addition of nitrogen and low dosages of glyphosate on flowering, nectar volume, sugar percentage and sugar composition of three selected plants of importance to pollinators (*Leucanthemum vulgare*, *Linaria vulgaris* and *Tanacetum vulgare*), abundant within most subplots and most treatments at the Kalø experimental plot. Glyphosate, however, has the potential to affect not only flowering and sugar characteristics of the nectar, but also the amino acids in nectar and pollen. The toxic activity of glyphosate works through blocking the synthesis of the aromatic amino acids, phenylalanine, tyrosine and tryptophan. In a study of 73 bee-plants, phenylalanine was the most commonly occurring amino acid in the nectars, and the content of phenylalanine was the factor best explaining the flower preferences of the pollinators in the field (Petanidou 1999). Glyphosate, therefore, might be expected to affect the amino acids within the plants and possibly also pollination.

Some of the necessary plant trait data has already been collected in existing databases, e.g. LEDA (Kleyer et al. 2008) and NOVANA (Nielsen et al. 2012) and preliminary results suggest that plant traits are useful for describing the gradual functional change in the grassland vegetation underneath hedgerows, where the neighbouring fields have been converted to organic farming practice. However, plant trait data that are linked to pollination and root structure are currently lacking. *The objective of this study is to quantify the effect of glyphosate and nitrogen treatments on selected plant and soil fauna traits and to quantify the causal relationships among these plant and soil fauna traits at the ecosystem level in a SEM (Fig. 1.1).*

It was only possible to address these ambitious research objectives because we have access to *"Kaløplottet"*, where simulated grassland vegetation is treated with both glyphosate and nitrogen in a factorial design with 120 subplots at levels that simulate drift from field edges. During the ten years the experiment has been running, significant effects of both glyphosate and nitrogen have been observed on vegetation composition, species richness and abundance and biomass of individual plant species (Holst et al. 2008, Damgaard et al. 2011, Strandberg et al. 2012, Damgaard et al. 2013, Damgaard et al. submitted-b). The design of the field-experiment and the performed scientific work during the ten year period enabled us to investigate the following hypotheses, which also are relevant for regulating the use of herbicides:

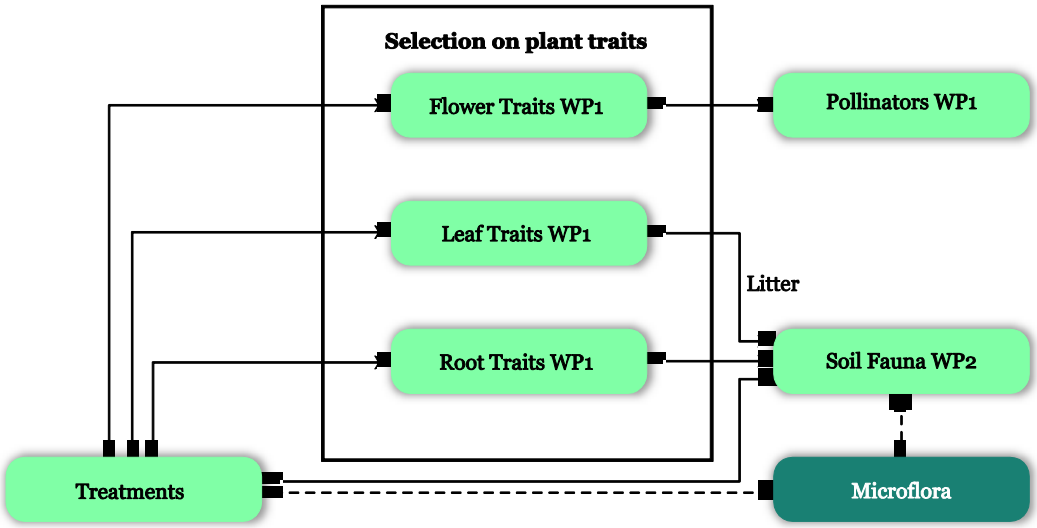

FIG. 1.1. OUTLINE OF THE RELATIONSHIPS BETWEEN THE NITROGEN AND GLYPHOSATE TREATMENTS AND THE STUDIES ECOSYSTEM FUNCTIONS AND STRUCTURES. THIS FIGURE WILL PROVIDE THE BACKBONE IN THE STRUCTURAL EQUATION MODEL. THE PROPERTIES INDICATED BY THE LIGHT GREY BOXES WILL BE QUANTIFIED BY SAMPLED DATA IN THE PROJECT. THE BLACK BOX "MICROFLORA" IS AN EXAMPLE OF AN IMPORTANT ECOSYSTEM COMPONENT THAT IS NOT QUANTIFIED IN THE PROJECT.

2. Materials and Methods

2.1 The Kalø field experiment

The effect of glyphosate and nitrogen and their interactions was studied at the experimental site "Kaløplottet". The Kalø field experiment was established in 2001 (Bruus Pedersen et al. 2004). The area selected was a former agricultural field on dry, nutrient poor sandy soil. The field laid fallow a couple of years prior to the start of the experiment in 2001. The field is quadrangular and surrounded by small parts of forest on two sides (south and west) and separated from the neighbouring fields by 5 meter broad hedgerows on the other sides. In 2001, the area was deep ploughed down to 60 cm to eliminate establishment from the soil seed bank and prepared for the experiment by harrowing and rolling. Thirty-one species were sown in spring 2001. The species selected were grassland species covering different life form strategies (CRS strategies sensu Grime 2001).

2.1.1 Experimental manipulations

The experimental manipulations were set up as a complete randomized block design with 10 replicates of each of the twelve treatments (Fig. 2.1.1.1). The treatments included 4 glyphosate treatments (0; 14.4; 72 and 360 g a.i./ha equal to 0, 1, 5 and 25% of label rate of 1440 g glyphosate/ha) and 3 nitrogen treatments (0, 25 and 100 kg N/ha). All plots received phosphorus (53 kg/ha), potassium (141 kg/ha), sulphur (50 kg/ha and copper (0.7 kg/ha) every year. The nitrogen was added as N27 CAN, calcium ammonium nitrate (NH_4NO_3+$CaCO_3$). The RoundupBio® formulation of glyphosate was used for the experiment. Each plot was 7 m x 7 m with a buffer zone of 1.5 m surrounding the plot. A buffer zone of 10 m separated the experiment from the surrounding vegetation. The buffer zones were also sown with the seed mixture. For the herbicide applications, spraying equipment for experimental applications was used. The beam was 3 m with 0.5 m between the nozzles that were Lurmark Lo-drift LD 015 Green nozzles with a pressure of 2.0 bars. The wind speed on the days selected for spraying was very low (0-2 m/s). There was no rain, neither was rain expected during the days following the day of spraying. Fertilizers were spread by hand. The plots were treated by glyphosate for the first time on 24th of August 2001, when the vegetation had become established at the plots. Since then, it has been treated with herbicide and fertilizer once every year in spring (mid-ultimo May) (Table 2.1.1.1).

Treatment/Sampling	2005	2006	2007	2012
Fertilizer application	12. May	15. May	15. May	24. May
Glyphosate spraying	30. May	30. May	7. June	28. May
Pre-treatment sampling	23.-25. May	24.-27. May	30. May- 5. June	
After treatment sampling	14.– 20. June	17.-22. June	21.-26. June	11.-12. June
End of season sampling	26.–31. August	25.-29. August	27.–31. August	

TABLE 2.1.1.1.
TIME OF HERBICIDE AND FERTILIZER TREATMENTS AND VEGETATION SAMPLINGS AT THE KALØ EXPERIMENTAL PLOT SHOWN FOR THE FOUR YEARS INCLUDED IN THE PRESENT STUDY. THE TREATMENT TIMES ARE ONLY SHOWN IN THE YEARS WHERE VEGETATION SAMPLING WAS PERFORMED.

5	1	7	11	9	3	7	5	10	2	12
4	2	3	10	3	2	11	4	4	4	11
9	4	12	9	12	9	1	1	8	10	10
10	9	4	5	11	1	9	8	6	6	9
6	3	2	2	5	10	8	2	12	9	8
2	8	6	4	1	8	3	9	7	8	7
12	7	10	6	6	11	10	3	11	11	6
11	5	1	3	7	7	2	11	9	5	5
8	11	8	12	10	5	5	7	3	7	4
7	10	9	1	2	4	12	6	1	1	3
3	6	11	7	4	12	4	10	2	3	2
1	12	5	8	8	6	6	12	5	12	1
J	I	H	G	F	E	D	C	B	A	

Experimental design
Plot size: 7×7 m
10 replicates of each of treatments
Label rate (=100%) was 1440 g a.i./ha

	N (kg/ha/year)	RoundupBio® (% of label rate)	RoundupBio® (g a.i./ha)
1	0	0	0
2	0	1	14.4
3	0	5	72
4	0	25	360
5	25	0	0
6	25	1	14.4
7	25	5	72
8	25	25	360
9	100	0	0
10	100	1	14.4
11	100	5	72
12	100	25	360

FIGURE 2.1.1.1.
EXPERIMENTAL DESIGN

2.2 Sampling programme

All soil samples were collected randomly within the centre of the plots using the design outlined in Fig. 2.2.1. More specific details on the sampling of specific biological variables are described in the relevant section below.

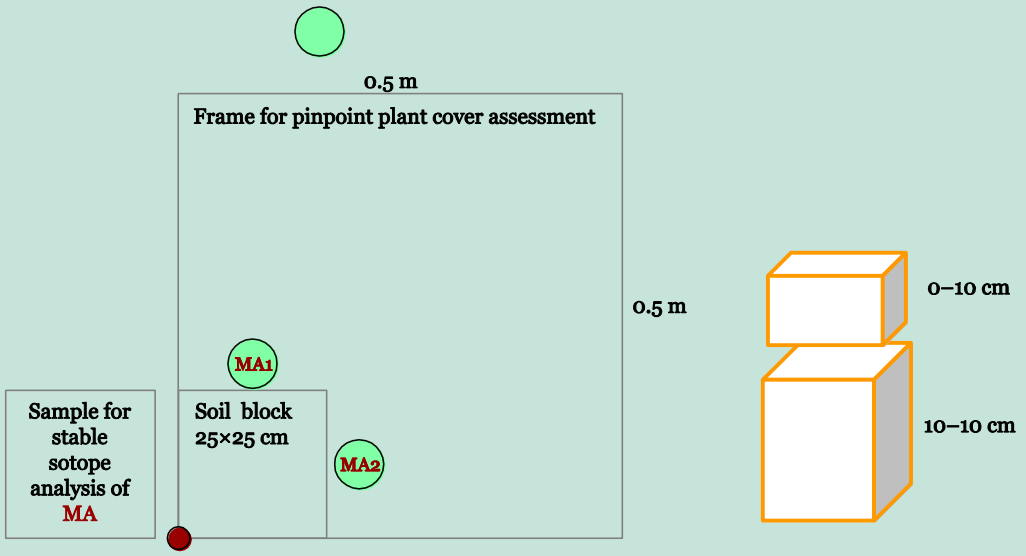

FIGURE 2.2.1.
SITUATION OF THE SOIL SAMPLES AND THE PIN-POINT FRAME WITHIN A PLOT. MA: MICROARTHROPOD SOIL CORE SAMPLE.

2.2.1 Measurements of cover and biomass of vascular plants at the Kalø experimental plot

Sampling of plant cover was made within 6 randomly selected 0.5m x 0.5m quadrates in each plot. During the years 2005-2007, only plots receiving the intermediate and highest concentrations of nitrogen, i.e. 25 and 100 kg N/ha, respectively, were sampled, whereas all plots were sampled in 2012. Plant cover was estimated within each quadrate by the pin-point (or point-intercept) method (Levy and Madden 1933, Kent and Coker 1992) using a horizontal frame with a 5x5 grid with the 25 intersections at a distance of 10 cm. At each intersection, a sharply pointed pin with a diameter of 0.5 mm was passed vertically through the vegetation. Nomenclature follows (Hansen 1991).

The pin-point cover data sampled in 2012 of the ten most common plant species was analysed by a generalized linear mixed effect model using R-INLA (Rue et al. 2009), where nitrogen and glyphosate were fixed factors, columns (=blocks) were random effects, and the error was assumed to be beta-binomially distributed (Damgaard 2009, 2012). The community weighted average trait values in 2012 were analysed in the same model, apart from the fact that the error was assumed to be normally distributed. Statistical inferences were based on the estimated 95% credibility interval.

2.2.2 Plant and root traits

Several above and below-ground plant traits were measured for the species at the Kalø experimental plot using standard methodologies (Cornelissen et al. 2003, Garnier et al. 2007, Stokes et al. 2009) except for inconspicuous rare species. Priority was given to measuring plant traits that are relevant for the objectives of the project, such as life history, flowering phenology, mode of pollination, root profile, root structure, and average root thickness (se Appendix 1).

Selected root traits were sampled for the four overall dominant species, i.e. *Festuca ovina*, *Elytria repens*, *Tanacetum vulgare* and *Euphorbia esula*. These included specific root length (SRL), fine root diameter, root depth distribution and 95% rooting depth. Generally, we sampled according to the protocols for standardised measurements of plant functional traits suggested by (Cornelissen et al. 2003).

2.2.3 Measuring pollination related traits and effects of addition of nitrogen and glyphosate on pollination

Study species

Plant traits of potential importance to pollination were recorded for two species of Asteraceae that were found in most plots and common within most treatments: *Leucanthemum vulgare* and *Tanacetum vulgare* (Table 2.2.3.1). The former has an early summer flowering season, while the latter is a late summer flowering species. Thus, *Leucanthemum* was expected to flower shortly after the time of herbicide application, while *Tanacetum* was only present as small vegetative shoots re-sprouting from the root system at the time of spraying.

Leucanthemum vulgare, Ox-eye Daisy, is a perennial herb native to Eurasia, and a common wildflower of temperate grasslands. The plant is tolerant to frost and drought, but has a moderate requirement for nitrogen. It grows as lateral rhizomes with erect, usually non-branched stems of 30-80 cm. Flower heads are mostly solitary and variable in size, 2.5-5.0 cm in diameter (Howarth and Williams 1968). They consist of a yellow disk of tiny (4 mm long), hermaphroditic yellow flowers, surrounded by white female ray florets, which consist of a long white ligule attached to a short corolla tube. The flowers are insect pollinated and attract a variety of insects, including Diptera, Hymenoptera, Coleoptera, Lepidoptera and Thysanoptera. Each flower head produces a large number of oblong achenes without a pappus; seeds are shed in August/September and disperse mainly by wind. The plant reproduces by seeds as well as vegetatively by rhizomes (Howarth and Williams 1968).

Tanacetum vulgare, Tansy, is an herbaceous perennial plant native to Eurasia. It is a common plant of field margins, roadsides, waste areas and grasslands. The plant grows as clusters of erect stems of 30-150 cm, which branch towards the top. Yellow button-like capitula are borne in dense terminal corymbs consisting of 10-70 capitula (Mossberg and Stenberg 2007). Capitula consist of tiny tubular yellow disk florets, the disk is 7-11 mm in diameter without ray florets (Mossberg and Stenberg 2007), flowers open from the periphery towards the centre, pollen is conspicuously yellow, and the flowers emit a characteristic scent (pers. obs). Flowers are hermaphrodite and pollinated by bees, flies, beetle and selfing. Capitula develop numerous tufted seeds, which are dispersed by wind or water.

Plant traits related to pollination

Treatments	Observation dates	0% glyphosate, 0 kg N/ha/year (treatment 1)	1% glyphosate, 0 kg N/ha/year (treatment 2)	5% glyphosate, 0 kg N/ha/year (treatment 3)	25% glyphosate, 0 kg N/ha/year (treatment 4)	0% glyphosate, 100 kg N/ha/year (treatment 1)	25% glyphosate, 100 kg N/ha/year (treatment 1)
Leucanthemum vulgare							
Phenology	12-06-2013-10-07-2013	x			x	x	x
	10-07-2013-05-08-2013	x	x	x	x		x
	05-08-2013-13-11-2013	x	x	x	x	x	x
Ray floret length	25-06-2013	x			x		x
	10-07-2013	x	x	x	x		x
Disk diameter	25-06-2013	x			x		x
	10-07-2013	x	x	x	x		x
Nectar							
Flower visitors	12-06-2013	x			x	x	x
Seed set		x	x	x	x		x
Tanacetum vulgare							
Phenology	10-07-2013-24-07-2013	x	x	x	x		x
	05-08-2013-13-11-2013	x	x	x	x	x	x
Disk diameter	05-08-2013 and 07-10-2013	x	x	x	x	x	x
Number of umbels/stem	05-08-2013 and 07-10-2013	x	x	x	x	x	x
Height at flowering	05-08-2013 and 07-10-2013	x	x	x	x	x	x
Nectar							
Flower visitors	29-07, 02-08, 05-08, 03-10, 07-10-2013	x	x	x	x	x	x
Seed set		x	x	x	x	x	x

TABLE 2.2.3.1.
PLANT TRAITS AND PLOTS (TREATMENTS) INCLUDED IN THE STUDY

In both plant species, we measured traits describing flower morphology, phenology, nectar reward, pollinator visitation and diversity, and seed set. Morphological traits, nectar sampling and pollinator observations were carried out during peak flowering of each of the two species in the treatments. This implies that in some cases, particularly the high pesticide treatments, some plots were sampled later than others, due to delayed flowering.

Phenology and abundance
For *Leucanthemum vulgare,* the number of open, pollen presenting flower heads per plot (excluding a buffer zone of 0.5 meters) was registered once every fortnight from the 12th of June 2013 (in plots of treatments 1, 4, 9 and 12, from July 10th treatment 1,2,3,4 and 12, from August 5th treatment 1,2,3,4,9 and 12). The flower head counts excluded a buffer zone of 0.5 meters from the edge of each plot (i.e. flower density was monitored in 6×6 meter plots).

For *Tanacetum*, the number of stems in flower, the number of completely withered stems, and the number of stems only with buds per plot (excluding a buffer zone of 0.5 meters) were registered once every two weeks from the 10th of July for treatments 1, 2, 3, 4, 12 (earliest flowering was 17 July), and from 1 August 2013 also in treatment 9. Flowering was monitored until the end of the growing season, coinciding with the first frost (13 November 2013). We defined capitula as flowering when pollen was presented. Stems without visible buds or flowers were not included in the counts. The flowering data was fitted to Gompertz growth curves.

Floral morphology
On June 25, 2013, we measured the diameter of the disk and length of three ray petals in each of 10 flowers of *Leucanthemum* per plot (or as many as possible in plots with <10 flowers) in treatments 1, 4 and 12, and on July 10 2013 in treatments 1, 2, 3, 4 and 12. Flower heads were selected at random, but preferentially from different parts of the plot, to sample different individuals. The three ray florets were selected at random, approximately equally spaced (in a Y-pattern, 2 o'clock, 6 o'clock and 10'o'clock) on the capitulum. Length of the ray petals was measured as the maximum length, including both the ligule and the short corolla tube. In the control treatment (treatment 1), we measured the length of an additional seven ray florets on July 10 to obtain a measure of variation in petal length of untreated flowers.

For *Tanacetum,* we counted the number of corymbs per flowering stem, the height of the stem at flowering (plants did not grow much after onset of flowering), and diameter of three flower heads per stem in 10 stems per plot. These stems were sampled at random, but preferentially from different spatial locations (clusters of *Tanacetum*) within the plot. For the diameter measurements, fresh, pollen presenting capitula were chosen, preferentially from the top umbel, but avoiding terminal capitula, which were sometimes larger. Some stems had one larger terminal flower head. If flower heads were in different stages of flowering, we chose those at peak flowering (presenting most pollen). In the untreated control, we measured the diameter of an additional 7 capitula in each of the 10 stems to assess the natural level of within-stem and among stem variation. Floral traits were measured during the peak flowering season, hence, plants in plots of high pesticide treatments (treatments 4 and 12) were measured in late season (7 October 2013) due to delayed flowering in these plots. Furthermore, we measured stem height at flowering and number of corymbs per stem for each of 10 stems in each plot.

Flower visitor observations
Flower visiting insects of *Leucanthemum* were observed on 12 June 2013 (at peak flowering of control plots) in all plots of treatments 1, 4, 9 and 12. A quadrate of 0.5×0.5 meters was placed in the densest patch of flowers within the plot and observed for 5 minutes. The number of flower heads in the quadrate was counted. The weather was sunny with a few clouds, light wind, and 20-23 °C.

The species and abundance of flower visiting insects of *Tanacetum* were recorded on 29 July 2013. Flower visitors of *Tanacetum vulgare* were observed in a "cluster" of stems (possibly same genetic individual), preferentially 10-15 stems within an observation area of 0.5*0.5m in each plot for 5 minutes (treatments 1, 2, 3, 4, and 12). Insects which could not be identified in the field were collected for later identification. The weather was sunny with few clouds, light wind, 27-29 degrees, and insect activity was high. For each observation plot (0.5*0.5m), the total number of stems and

the number of flowering stems were recorded, in addition to number of pollen presenting flower heads and non-flowering flower heads (buds) per flowering stem. Flowering ranged from beginning to peak flowering period.

Collected insect specimens were identified by the following taxonomists: Rune Bygebjerg (Diptera, Syrphidae), Boy Overgaard Nielsen (other Diptera), Jørgen A. Axelsen (Coleoptera and Lepidoptera), Henning Bang Madsen (Apoidea, Hymenoptera).

Statistical analysis

The effect of nitrogen and glyphosate on the flowering phenology was investigated using the Gompertz growth model (Seber and Wild 1989),

$$f(t) = \alpha \exp\left(-\exp\left(-\kappa(t-\gamma)\right)\right) \quad (1)$$

where $f(t)$ is the cumulative flowering at time t, α is the asymptotic number of cumulative flowering stems the maximum growth rate is $\kappa\ \alpha/e$, and γ is the time where the growth curve has the point of inflection. The three parameters in the Gompertz growth model (1) were all generalized into linear functions of the nitrogen and glyphosate level, i.e.

$$h(\theta) = \theta_0 + \theta_N\, n + \theta_G\, G + \theta_{NG}\, N * G \quad (2),$$

where N is the nitrogen level and G is the glyphosate level. Thus, there were a total of 12 parameters that were fitted to the data using a Bayesian MCMC approach (Metropolis-Hastings algorithm), where the cumulative flowering data were assumed to be Poisson distributed and all parameters were assumed to have a uniform prior distribution. The joint posterior distributions were sampled from 50,000 MCMC iterations with a burn-in period of 10,000 iterations. Statistical inferences were based on the 95% credible interval of the marginal posterior distributions. The MCMC iterations were performed using *Mathematica* (Wolfram 2013).

Plant height, diameter of capitulum, pollinator visitation rate, pollinator diversity and seed weight per capitulum were analyzed in mixed effect models using the *lme* procedure in R with blocks (treatments are randomized within blocks in the set-up) in the case of plant height, pollinator visitation rate and diversity or plant individual nested within block in the case of capitulum diameter and seed weight per capitulum as the random effect(s).

2.2.4 Soil Fauna

The experimental area was sampled in each plot for microarthropods, i.e. collembolans, mites and earthworms on October 1-5, 2012. Microarthropod samples were collected with a soil corer, diameter 5.8 cm and to a depth of 5.5 cm. From every plot, a bulk sample of approx. 20x20 cm^2 of the top 5 cm of the soil was collected for analysis of stable isotopes (Fig. 2.1.2). Collembolans were identified to lowest taxonomic level and separated for characterisation of isotopic signatures, $\delta^{13}C$ and $\delta^{15}N$, selected groups of soil mites and insects encountered in the bulk sample.

A new collembolan and earthworm trait database has been established and contains a range of morphological and ecological traits. It is accessible from www.SoilBioStore.AU.dk. See Appendix 2 for a list of the traits for which data has been collected. The trait database is used to create trait scores by multiplying the abundance matrix with the trait matrix (se Appendix 2).

Soil blocks of 25x25x30 cm^3 for earthworm abundance and biomass were sampled in half the blocks (A, C, E, G, I) October 5-9 and the second half (B, D, F, H, J) from October 25-31 2012. Only the top 10 cm were used for abundance and biomass analyses, as barely any worms were found from 10-30 cm. The soil blocks were hand-sorted, and earthworms were collected and identified to species, while fresh biomass was determined species-wise per block.

The biomasses and abundances of earthworms obtained in WP2.2 has been subject to a *mixed* modelling analysis taking into account the design and treatment structure of the field plot layout.

The δ signatures are included as traits in the established earthworm trait databases (Appendix 2).

2.2.5 Soil physiscs

The former characterisation of the soil texture as described by Pedersen *et al.* (2004) was reassessed by a small selection of representative plots for extreme and average soil textures, spanning coarse sand to clayey sand (JB 1 to 4): A6 , E10 , G1 , D6 , A12 and a sample at depth 40 cm. These samples were analysed at OK Lab, Viborg, Denmark, according to Danish standards (Plantedirektoratet 1994). All these new analyses were categorised as coarse sand, hence, we could not confirm any of the other soil texture types, i.e. JB>1, previously found at the study location (Table 2.2.5.1). However, the small number of analyses confirmed the average soil type over the whole experimental area. The humus content had apparently increased from 0.6 [0.6-0.7]% to 1.4 [1.2-1.5]%, as expected from the change to grassland from agricultural land. In every second block, A, C, E, G, I, we also determined the soil organic matter % by Loss on Ignition (LOI) and total C %. It was 3.9 [3.4-4.3] and 1.8 [1.5-2.1] without any statistically significant relationship with the treatment factors.

Plot	Coarse sand	Fine sand	Silt	Clay	Humus
A6	56.8	35	2.3	4.3	1.59
E10	42.2	49.9	2.0	4.3	1.60
G1	66.9	27.9	1.2	2.9	1.19
D6	73.9	20.0	2.0	2.9	1.19
A12	73.7	22.9	0.4	2.2	0.78
Sample <40_cm	54.9	36.5	2.7	4.1	1.78

TABLE 2.2.5.1.
ANALYSES OF SOIL TEXTURE IN A SUBSET OF PLOTS REPRESENTING THE SPAN OF SOIL TYPES AT THE STUDY LOCATION.

2.3 Stable isotopes

Soil, fresh plant material (roots and shoots), litter, microarthropods and earthworms were sampled for stable isotope analysis and dried at 60° C for maximum 24 h or until constant weight. For each of these types of material, we had 60, 73, 56, 49 and 75 samples for analyses of isotopes, $^{14}N/^{15}N$ and $^{12}C/^{13}C$, respectively. The analyses were performed at UC-Davis Stable Isotope Facility, California. A representative sample of the soil from cores 0-5.5 cm depth for microarthropods was sieved through 2 mm mesh and used to indicate the isotope ratios for total soil organic matter. Every second block was sampled for this purpose. All visible non-soil organic matter was removed from the soil, including roots and plant remains. For the dominant plant species, a representative sample of roots was collected from the upper 0-10 cm of a soil block and separated into species. A soil block was dug with a spade in every second block, resulting in 60 soil blocks. Microarthropods were extracted alive on to plaster/charcoal, and a sufficient number of individuals, typically consisting of pooled individuals, e.g. between 1 and 120 adult specimens based on body size of the species, are needed to obtain sufficient material for $^{15}N/^{13}C$ analysis. In all cases, microarthropods had to be collected across the blocks to have sufficient biomass, and block variation was not determined. Earthworm isotopic signatures were determined for individual adult and juvenile specimens collected across blocks and treatments. For *A. longa* and *L. rubellus,* only one specimen was available per species from the whole experiment.

Comparison of treatments for stable isotopes were done by a three-way ANOVA with the factors N, Gly and taxon, where the taxon factor was a range of soil invertebrate or plant species. All ANOVA's and mixed models for soil invertebrate, trait and stable isotope data were performed with PROC MIXED of SAS/STAT (SAS-Institute-Inc. 2013). The block was included as a random factor in the model, while species and the treatment factors glyphosate and nitrogen fertilizer were included as fixed factors.

2.4 Structural equation modelling

The selective pressure of nitrogen and glyphosate on plant traits (Appendix 1) and the consequent cascading effect on soil fauna abundance and traits (Appendix 2) was modelled using structural equation modelling (SEM).

The advantage of using SEM is among other things that the hypothesized causal mechanisms are specified in graphical models, which enable tests for conditional independence and estimation of direct and indirect effects (Grace et al. 2010). The examined structural equation models were specified according to prior information and corroborated hypotheses on the causal relationships among the ecological variables using the R-package "lavaan" version 0.5-15 (Rosseel 2012). The used ecological variables were transformed in order to be of approximately the same order of magnitude and approximately normally distributed (according to a graphical inspection).
Only traits where substantiated ecological knowledge suggests causal connections to the effects of the treatments were included in the SEM. There are two main approaches on how to specify the causal relationships in a structural equations model (SEM): i) an *a priori* approach, where the causal relationships in the model are established from existing ecological knowledge that is assessable through the literature, or ii) an empirical approach, where the modelled relationships primarily are guided by the actual observations in the experiment at hand and insignificant causal relationships are omitted from the model in an iterative process. In this study, we have chosen the *a priori* approach and not omitted non-significant causal relationships. This choice is motivated by the notion that the removal of non-significant, but otherwise firmly established, causal relationships will change the overall structure of the SEM in an unknown way and, consequently, too much weight would be given to possible type II errors (see also Hooper et al. 2008).

Some ecological variables were not determined for all plots. This was especially the case for root biomass, which was only determined in 60 of the 120 plots. In order to apply the SEM approach, the missing values were simulated using the lavann procedure: missing="ml". The qualitative robustness of the resulting SEM was checked by comparing with the SEM fitted from the actual dataset where all plots with missing data were omitted.

3. Results

3.1 Effects of nitrogen and glyphosate on plant species composition

The nitrogen and glyphosate treatments had significant effects on the species composition at the experimental plot. The cover of the most abundant plant species, including four grasses, i.e. *Festuca ovina*, *Elytrigia repens*, *Agrostis gigantea*, *Agrostis capillaris*, and five dicotyledons, *Tanacetum vulgare*, *Euphorbia esula*, *Leucanthemum vulgare*, *Hierachium pilosella* and *Linaria vulgaris*, varied with treatment (Table 3.1.1, Fig. 3.1.1). Generally, species cover decreased with increasing glyphosate doses. Cover of *F. ovina* and *E. esula* form the only exceptions. For these species, the cover increased with increasing dose of glyphosate. Whereas the cover of *F. ovina* was high in 2005 and has continued to increase in plots treated with glyphosate, the cover of *E. esula* was low at that time, but has increased over the years. Increasing nitrogen, generally, resulted in increasing total plant cover and, especially, in increased biomass which was already estimated in a previous project (Strandberg et al. 2012). A few species adapted to nutrient poor conditions, especially *F. ovina* and *H. pilosella* form exceptions. Additionally, increasing glyphosate doses, especially in combination with the highest level of nitrogen, which is exactly the kind of condition found in many habitats adjacent to conventional agricultural fields, increased the cover of bare soil significantly. This corresponds well with an increasing fraction of annual species within these plots. All annuals were weed species such as *Polygonum aviculare*, *Viola arvense*, *V. tricolora* and *Erodium cicutarium* traditionally found in agricultural fields under annual crop rotation.

Species	Nitrogen		Glyphosate		Nitrogen*Glyphosate		Overdisp.
	Mean	SD	Mean	SD	Mean	SD	
Bare Soil	-0.0166	0.0025	0.0290	0.0067	0.0004	0.0001	0.0985
Festuca ovina	-0.0417	0.0023	0.0103	0.0070	0.0013	0.0001	0.4508
Elytrigia repens	0.0419	0.0021	-0.1037	0.0187	-0.0006	0.0002	0.4616
Tanacetum vulgare	0.0022	0.0018	-0.0352	0.0101	0.0002	0.0002	0.4072
Euphorbia esula	0.0047	0.0022	0.0035	0.0109	0.0003	0.0002	0.5244
Leucanthemum vulgare	-0.0233	0.0032	-0.0885	0.0152	0.0012	0.0003	0.2958
Agrostis gigantea	-0.0312	0.0038	-0.1108	0.0171	0.0014	0.0003	0.3038
Hieracium pilosella	-0.0661	0.0100	-0.0610	0.0137	0.0020	0.0005	0.4539
Linaria vulgaris	-0.0140	0.0042	-0.0014	0.0129	0.0008	0.0002	0.0957
Agrostis capillaris	-0.0249	0.0045	-0.1276	0.0288	0.0011	0.0005	0.3532

TABLE 3.1.1.
MEAN AND STANDARD DEVIATION OF THE ESTIMATED POSTERIOR DISTRIBUTION OF THE EFFECTS OF NITROGEN, GLYPHOSATE, AND THE INTERACTION BETWEEN NITROGEN AND GLYPHOSATE ON THE SAMPLED COVER IN 2012 OF THE TEN MOST COMMON PLANT SPECIES AS WELL AS THE ESTIMATED SPATIAL OVERDISPERSION OF THE SPECIES (STATISTICAL OVER DISPERSION RELATIVE TO RANDOM EXPECTATIONS). IF THE ESTIMATED 95% CREDIBILITY INTERVAL DOES NOT INCLUDE ZERO, WHICH INDICATES A SIGNIFICANT EFFECT, THEN THE MEAN EFFECT IS SHOWN AS BOLD.

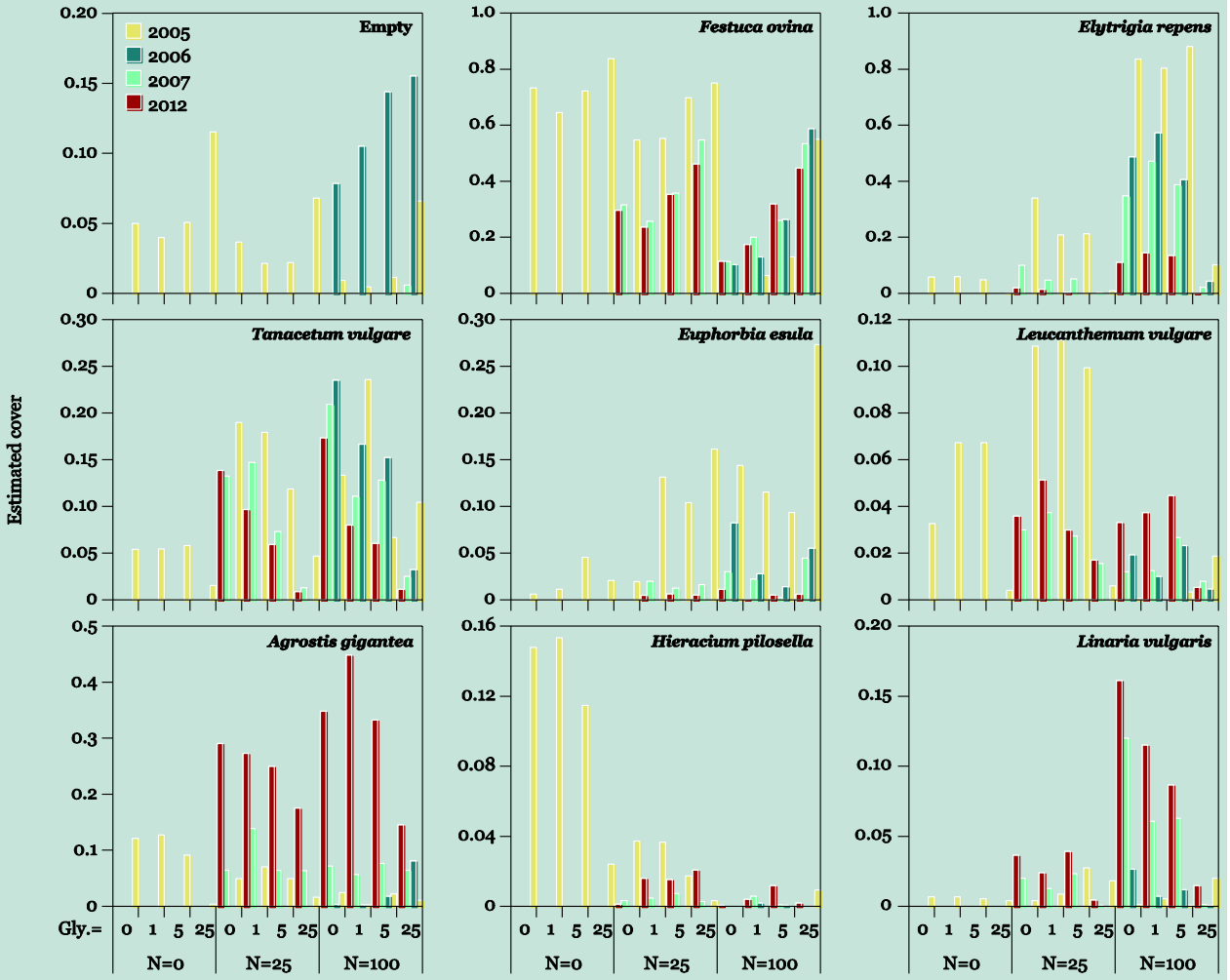

FIGURE 3.1.1.
THE ESTIMATED COVER OF THE MOST COMMON PLANT SPECIES AND UN-VEGETATED (BARE) SOIL (EMPTY BELOW) AT THE DIFFERENT TREATMENTS.

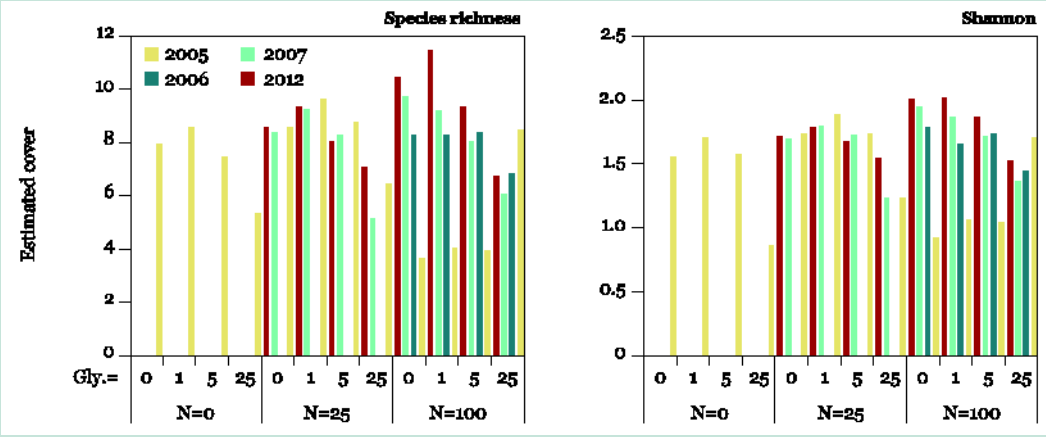

FIGURE 3.1.2.
PLANT DIVERSITY MEASURED AS SPECIES RICHNESS AND SHANNON DIVERSITY INDICES FOR ALL TREATMENTS.

Generally, increasing nitrogen as well as glyphosate dose reduced plant diversity measured either as species richness or by the Shannon diversity index (Fig. 3.1.2). However, we found a positive interaction effect for both diversity measures. This presumably is a consequence of the increasing number of annuals (see below) found at the highest concentrations of both treatments, and the calculated evenness indices, i.e. how close the cover values are for all species within a treatment, also support this (Fig. 3.1.3).

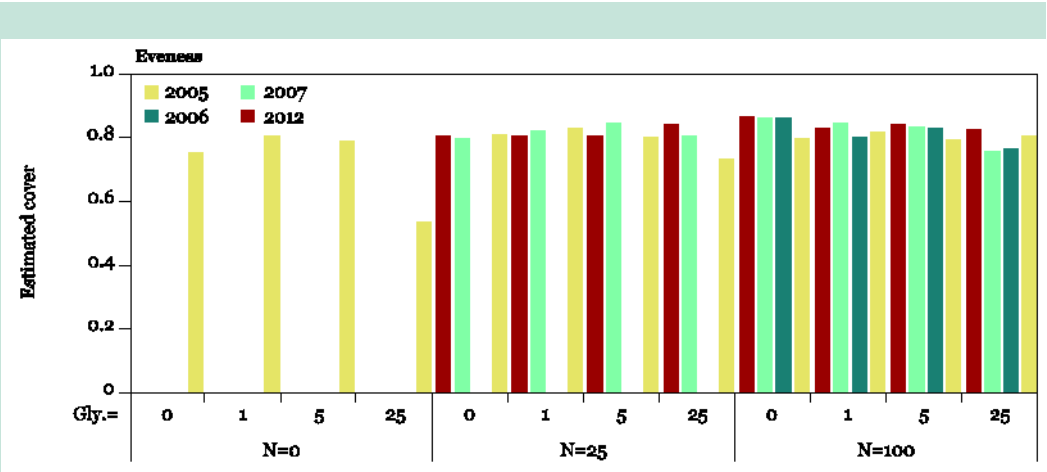

FIGURE 3.1.3.
SPECIES EVENNESS REPRESENTED BY THE PIELOU EVENNESS INDEX FOR ALL TREATMENTS.

3.2 Effects of nitrogen and glyphosate on plant traits

3.2.1 Above-ground plant traits

Generally, nitrogen increased the Ellenberg indices (se Appendix 1) that vary between 1 and 9 except for Ellenberg F (humidity), which has values between 1 and 12. Readily understandable is the increase of Ellenberg N (nitrogen level) from about 3 (indicating nutrient poor conditions) for plots receiving no nitrogen up to between 5 and 6 (indicating a relatively high nitrogen level) for plots receiving 100 kg N/ha. It is more difficult to explain the significant increases in Ellenberg T (gives an indication of latitude) and Ellenberg K (continentality). The significant increase in Grimes C (competitiveness) and decrease in Grimes S (stress tolerance) with increasing nitrogen level are also directly explainable, as the more nutrient rich condition favours species that are highly competitive and less adapted to stressful conditions, such as *Elytrigia repens*.

In contrast, plots receiving increasing doses of glyphosate the Ellenberg indices generally decrease, except for Ellenberg T, which increases. Whereas it may be difficult to explain these changes, the increase in stress tolerance (Grime S) and annual life form with increasing glyphosate doses are directly explainable, as the herbicide acts as a stress factor and results in more bare soil where annual weeds establish. However, it is noteworthy that increasing glyphosate doses result in decreasing Grimes R (ruderal strategy). The ruderal strategy thrives on disturbed areas and the increasing incidence of stress tolerance, and concomitant decrease in importance of the ruderal strategy suggests that glyphosate acts on the plant community more as stress factor than as a disturbance.

Plant/canopy height increases with increasing nitrogen level and decreases with increasing glyphosate dose. However, plant height may not be considered as a plant trait, as it changes for the individual plant species with environmental conditions, and plant height is also used as effect measure (endpoint) in standard tests under the risk assessment of pesticides.

A number of the traits, i.e. leaf mass, life size, specific leaf area (SLA) and leaf dry matter content (LDMC), are, to some extent, related. SLA is defined as the ratio of leaf area to leaf dry mass, which decreases with increasing glyphosate. As SLA also decreases, this indicates that plants with small and thick leaves become more important with increasing glyphosate dose. LDMC, which increases with increasing glyphosate dose, is defined as the ratio of leaf dry mass to leaf fresh mass. Leaf thickness (LT) can also be estimated as (leaf area x leaf fresh mass)$^{-1}$ (Vile et al. 2005).

Trait	Nitrogen		Glyphosate		Nitrogen*Glyphosate	
	Mean	SD	Mean	SD	Mean	SD
Ellenberg L	0.00038	0.00023	-0.00124	0.00108	0.00003	0.00002
Ellenberg T	0.00410	0.00035	0.01017	0.00211	-0.00012	0.00003
Ellenberg K	0.03304	0.00090	-0.00880	0.00423	-0.00099	0.00007
Ellenberg F	0.00033	0.00154	-0.02242	0.00717	0.00001	0.00012
Ellenberg R	0.03018	0.00182	-0.02986	0.00696	-0.00075	0.00013
Ellenberg N	0.04449	0.00133	-0.04561	0.00619	-0.00108	0.00010
Grime C	0.06405	0.00227	-0.06994	0.01310	-0.00124	0.00021
Grime S	-0.05847	0.00526	0.12490	0.01374	0.00131	0.00030
Grime R	0.01252	0.00107	-0.02916	0.00620	0.00003	0.00010
Annual	-0.00001	0.00005	0.00050	0.00021	0.00000	0.00000
Seed mass	0.01543	0.00089	0.01355	0.00417	-0.00044	0.00007
SLA	0.09786	0.00368	-0.13874	0.01725	-0.00263	0.00029
Canopy height	0.00831	0.00025	-0.00686	0.00116	-0.00021	0.00002
LDMC	-0.13813	0.03084	1.38672	0.14386	-0.00551	0.00243
Leaf mass	0.83508	0.07139	-1.06143	0.33304	-0.00935	0.00563
Leaf size	22.72190	1.43657	-21.66734	6.52141	-0.39422	0.11187
Dicotyledons	-0.00042	0.00027	-0.00529	0.00125	0.00011	0.00002

TABLE 3.2.1.1.
MEAN AND STANDARD DEVIATION OF THE ESTIMATED POSTERIOR DISTRIBUTION OF THE EFFECTS OF NITROGEN, GLYPHOSATE, AND THE INTERACTION BETWEEN NITROGEN AND GLYPHOSATE ON THE COMMUNITY WEIGHTED AVERAGE TRAIT VALUE IN 2012. IF THE ESTIMATED 95% CREDIBILITY INTERVAL DOES NOT INCLUDE ZERO, WHICH INDICATES A SIGNIFICANT EFFECT, THEN THE MEAN EFFECT IS SHOWN AS BOLD.

FIGURE 3.2.1.1.
THE COMMUNITY WEIGHTED AVERAGE TRAIT VALUE AT THE DIFFERENT TREATMENTS. SEE APPENDIX 1 FOR UNITS.

3.2.2 Root traits

The root trait analysis is based on samples outside the treated plots from the upper 30 cm of the soil profile (0-10 cm and 10-30 cm, respectively) as 90-95 percent of the root biomass of forbs and grasses, normally, is found here (Schenk and Jackson 2002). However, the root systems of the four species, i.e. *Elytrigia repens*, *Festuca ovina*, *Euphorbia esula* and *Tanacetum vulgare*, are relatively different. The first three species have shallow roots with a maximal root depth of 40-45 cm and 90-98 percent of the root biomass in the upper 10 cm (Table 3.2.2.1.), whereas *T. vulgare* roots penetrate to a depth of about 1 meter, although the vast majority of the root biomass (85 %) is found in the upper 10 cm. *Elytrigia repens* has an extensive system of horizontal structural roots that ensures the rapid spread of this species through the soil and fine roots that ensure uptake of water and nutrients. For this species, structural roots make up about half of the root biomass. *Festuca ovina* has a dense fibrous root with 98 % of the root biomass (dry weight) found in the upper 10 cm. *Euphorbia esula* and *Tanacetum vulgare* have coarse, respectively, horizontal or vertical roots with relative few fine roots, and coarse roots make up a large part of the biomass, especially for *E. esula,* accounting for 70-99 % of the root biomass (dry weight).

	Soil depth 0-10 cm			Soil depth 10-30 cm		
Root depth distribution [g/m³]	Fine roots	Larger roots	total	Fine roots	Larger roots	total
Elytrigia repens	1891.5 ± 564.1	1591.1 ± 173.6	3482.7 ± 911.8	23.3 ± 19.3	92.8 ± 29.4	116.1 ± 48.2
Festuca ovina			9772.1 ± 3128.1			113.2 ± 61.2
Euphorbia esula	18.0 ± 9.6	517.3 ± 208.0	535.4 ± 216.4	2.6 ± 1.0	52.8 ± 27.6	55.4 ± 27.8
Tanacetum vulgare			598.2 ± 240.1			98.8 ± 26.3

TABLE 3.2.2.1.
ROOT DEPTH DISTRIBUTION (MEAN ± S.E.), I.E. DRY ROOT BIOMASS/VOLUME SOIL, FOR *ELYTRIGIA REPENS*, *FESTUCA OVINA*, *EUPHORBIA ESULA* AND *TANACETUM VULGARE* ESTIMATED FOR SOIL DEPTHS OF 0-10 AND 10-30 CM. FOR *E. REPENS* AND *E. ESULA* WE DISTINGUISHED BETWEEN FINE AND LARGER ROOTS.

Whereas most of the measured root traits varied considerably, the diameter of fine roots varied much less, both between individual plants and between topsoil (0-10 cm) and deeper layers (10-30 cm) (Table 3.2.2.2).

Diameter of fine roots [mm]	Soil depth	
	0-10 cm	10-30 cm
Elytrigia repens	0.27 ± 0.03	0.27 ± 0.03
Festuca ovina	0.18 ±0.03	0.18 ± 0.01
Euphorbia esula	0.33 ± 0.02	0.36 ± 0.02
Tanacetum vulgare	0.41 ± 0.09	0.23 ± 0.06

TABLE 3.2.2.2.
DIAMETER OF FINE ROOTS (MEAN ± S.E) FOR *ELYTRIGIA REPENS*, *FESTUCA OVINA*, *EUPHORBIA ESULA* AND *TANACETUM VULGARE*

The specific root lengths (SLR) measured for *Elytrigia repens* and *Festuca ovina* (Table 3.2.2.3) are comparable with SLR for others grass (Fort et al. 2012).

SRL [m/g]	Soil depth	
	0-10 cm	10-30 cm
Elytrigia repens	263 ± 8.9	197 ± 11.7
Festuca ovina	187 ± 9.1	164 ± 9.7
Euphorbia esula	*	*
Tanacetum vulgare	*	*

*THE COMBINED ROOT LENGTH FOR *EUPHORBIA ESULA* AND *TANACETUM VULGARE* COULD NOT BE DETERMINED AND SLR WAS NOT CALCULATED.

TABLE 3.2.2.3.
SPECIFIC ROOT LENGTHS (SLR) (MEAN ± S.E.) FOR *ELYTRIGIA REPENS*, *FESTUCA OVINA*, *EUPHORBIA ESULA* AND *TANACETUM VULGARE*.

3.3 Effects of nitrogen and glyphosate on pollination

Glyphosate and nitrogen had significant effects on the total number of flowers as well as when the flowers were produced, measured by the asymptotic number of cumulative flower stems and time of the inflection point of the growth curve, respectively (eqn. 1). The asymptotic number of cumulative flower stems, as measured by α in a Gompertz growth curve, was significantly affected by both nitrogen and glyphosate (Table 3.3.1). There were negative interaction effects of nitrogen and glyphosate and glyphosate alone on the asymptotic number of cumulative flower stems, whereas nitrogen without glyphosate had a positive effect on the asymptotic number of cumulative flower stems. Thus, glyphosate application generally lowered flower abundance, while nitrogen alone elevated flower abundance of *Tanacetum vulgare*. Furthermore, there were positive interaction effects of nitrogen and glyphosate and glyphosate alone on the time of the inflection point of the growth curve, γ, whereas nitrogen without glyphosate had no significant effect on the time of the inflection point. It is remarkable that glyphosate alone was estimated to postpone the inflection point with 1.5 days for every % application of glyphosate.

Parameter	Effects	2.5%	50%	97.5%	P(> 0)
κ_0	control	0,073562	0,075091	0,076012	1
κ_N	nitrogen	0,007085	0,007702	0,008131	1
κ_G	glyphosate	-0,00127	-0,00115	-0,001	0
κ_{NG}	nitrogen*glyphosate	-0,00033	-0,00032	-0,00029	0
α_0	control	469,7034	473,5856	477,3245	1
α_N	nitrogen	5,953613	6,041555	6,128734	1
α_G	glyphosate	-16,9596	-16,7746	-16,588	0
α_{NG}	nitrogen*glyphosate	-0,10248	-0,09172	-0,07705	0
γ_0	control	215,5807	215,887	216,2074	1
γ_N	nitrogen	-0,00422	-0,00099	0,002161	0,304944
γ_G	glyphosate	1,500467	1,512868	1,556504	1
γ_{NG}	nitrogen*glyphosate	0,00617	0,007375	0,008721	1

TABLE 3.3.1.
MARGINAL POSTERIOR DISTRIBUTIONS OF THE PARAMETERS K, A, AND Γ OF THE GOMPETZ CURVES FOR FLOWERING OF *TANACETUM VULGARE*. THE PARAMETERS IN THE GOMPERTZ GROWTH MODEL, K, A, AND Γ, ARE GENERALIZED AS SHOWN IN EQN. (2), WHERE THE SUBSCRIPT 0 DENOTES THE VALUE OF THE GROWTH PARAMETER IN THE UNTREATED CONTROL, THE SUBSCRIPT *N* DENOTES THE EFFECT OF NITROGEN, *G* THE EFFECT OF GLYPHOSATE, AND *N*G* THE INTERACTION EFFECT. EXCEPT FOR Γ_N, ALL PARAMETERS WERE SIGNIFICANTLY DIFFERENT FROM ZERO (IF $0.05 < P < 0.95$ THE EFFECT IS NOT SIGNIFICANT).

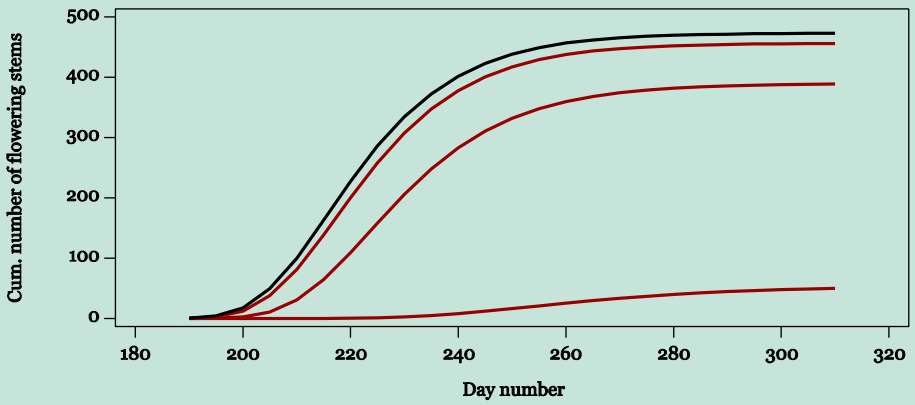

FIGURE 3.3.1.
GOMPERTZ GROWTH FLOWERING CURVES OF *TANACETUM VULGARE* FOR GLYPHOSATE TREATMENT WITH NO NITROGEN ADDED. BLACK CURVE: UNTREATED CONTROL, RED CURVES (FROM TOP): APPLICATION OF 1%, 5%, 25% GLYPHOSATE. FLOWERING CURVES INCLUDE PARAMETER UNCERTAINTY (LINES ARE SHOWN WITH 95% CREDIBLE INTERVALS). NOTE THAT THE UNCERTAINTY IS VERY SMALL.

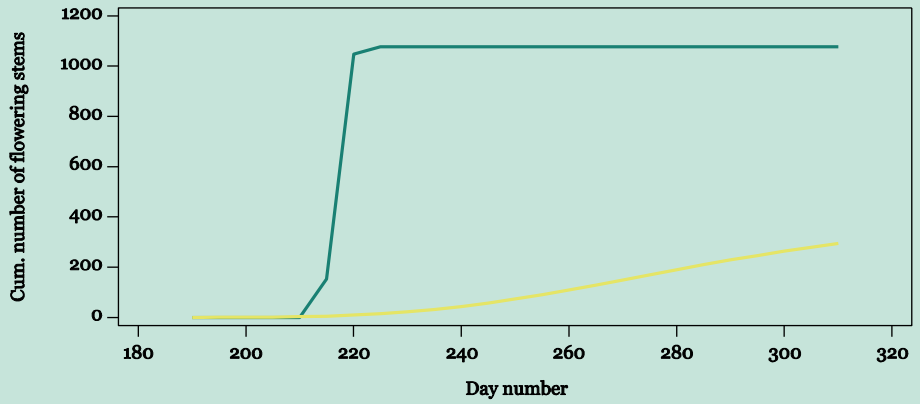

FIGURE3.3.2.
GOMPERTZ GROWTH FLOWERING CURVES FOR *TANACETUM VULGARE* IN TREATMENTS WITH 100 KG N/HA/YEAR AND 0% GLYPHOSATE (BLUE CURVE) AND 25% GLYPHOSATE (GREEN CURVE).

A mixed model analysis with plant individuals with block as random effects and treatments as fixed effects showed that the diameter of capitula did not differ between different levels of treatments by glyphosate and nitrogen (although N had a marginally positive effect (estimate = 0.004983, SE = 0.00254618, df = 237, t = 1.957165, P = 0.0515)). This may be due to the large variation explained by the random effect plants within blocks (59% of variance).

On the other hand, mixed model analysis with blocks as random effects and treatments as fixed effects showed that visitation rate (visits per stem per minute) was significantly negatively affected by glyphosate (estimate = -0.01113137, SE = 0.00481037, df = 48, t= -2.314039 $P < 0.05$), and marginally significantly positively affected nitrogen (estimate = 0.00228970, SE = 0.00115803 df = 48, t = 1.977242, P = 0.0538). The interaction effect was not significant ($P > 0.05$). Diversity (number of flower-visitor species) was significantly negatively affected by glyphosate, while no effect of nitrogen was found. However, a significantly positive interaction effect of glyphosate and nitrogen was found. Variations in both visitation rate and flower visitor diversity were explained mainly by the treatments (100% and 85%, respectively). Flower-visitor activity was high in flowering plots in July/August, and Tanacetum was visited by a range of different flower-visiting insects belonging to Diptera, Hymenoptera, Lepidoptera and Coleoptera. In particular, a wide range of syrphid flies visited the flowers (Table 3.3.2). In October, however, only Diptera was present (Fig. 3.3.3).

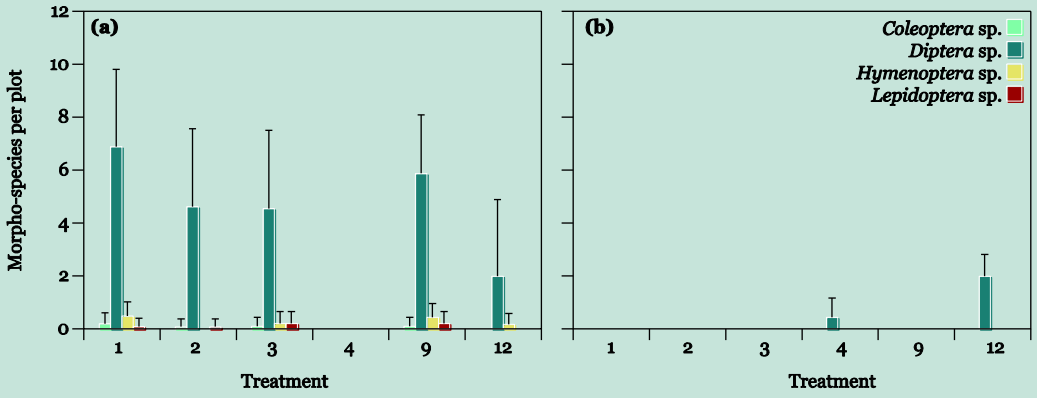

FIGURE 3.3.3.
NUMBER OF MORPHO-SPECIES OBSERVED VISITING FLOWERS OF *TANACETUM VULGARE* DURING 5 MINUTE OBSERVATION TRIALS IN 0.5*0.5M SUB-PLOTS IN EACH PLOT IN TREATMENT 1, 2, 3, 4, 9 AND 12.

Insect flower-visitors of *Tanacetum vulgare* (Asteraceae)

Order	Family	Species
Coleoptera	Coccinellidae	*Calvia quatuordecimguttata?*
		Coccinella septempunctata
	Nitidulidae	*Meligethes sp.*
Diptera	Anthomyiidae	*Anthomyiidae sp.*
	Calliphoridae	*Lucilia bufonivora*
		Lucilia sp.
		Melinda gentilis
	Conopidae	*Conops flavipes*
	Muscidae	*Muscidae sp.*
	Sarcophagidae	*Perretia nigriventris*
		Sarcophaga sp.
		Sarcophila latifrons
	Syrphidae	*Cheilosia vernalis female*
		Episyrphus balteatus
		Eristalis arbustorum
		Eristalis intricarius
		Eristalis lineata
		Eristalis sp.
		Eristalis tenax
		Eristalis tenax
		Eupeodes corollae
		Helophilus hybridus
		Plathycheirus albimanus
		Scaeva pyrastri
		Sericomyia silentis
		Sphaerophoria scripta
		Syritta pipiens
		Syrphus vitripennis
	Tachinidae	*Tachina fera*
		Tachinidae sp.
Hymenoptera	Andrenidae	*Andrena nigriceps*
	Apidae	*Bombus lapidarius*
		Bombus lucorum
	Colletidae	*Colletes fodiens*
	Formicidae	*Formicidae sp.*
	Halictidae	*Halictus sp.*
	Ichneumonidae	*Ichneumonidae sp.*
Lepidoptera	Hesperiidae	*Thymelicus lineola*
	Nymphalidae	*Maniola jurtina*
	Pieridae	*Pieris rapae*
		Pierus napi
	-	*microlepidoptera*

TABLE 3.3.2.
LIST OF INSECT FLOWER-VISITORS OBSERVED ON *TANACETUM VULGARE* FLOWERS IN JULY AND AUGUST

*G*ompertz curves fitted to phenological data for *Leucanthemum vulgare* showed that α, the asymptotic number of cumulative flowers, decreased significantly relative to the untreated control both when adding nitrogen and glyphosate, while a positive interaction effect was found (Table 3.3.3). However, only very few and stunted plants were found in the plots treated with 25% glyphosate. In accordance with *Tanacetum vulgare,* the inflection point γ of the flowering curves indicated that nitrogen had no significant effect, while glyphosate significantly postponed the

inflection point (for 21.5 days for every % application of glyphosate). Only a small interaction effect between glyphosate and nitrogen was found.

Parameter	Effects	2.5%	50%	97.5%	P(> 0)
κ_0	control	0,064561	0,066737	0,069262	1
κ_N	nitrogen	-0,0398	-0,03549	-0,03202	0
κ_G	glyphosate	-0,01189	-0,01139	-0,01096	0
κ_{NG}	nitrogen*glyphosate	-0,00026	0,000314	0,000757	0,708493
α_0	control	961,0995	973,6861	986,9825	1
α_N	nitrogen	-9,81997	-9,69042	-9,56668	0
α_G	glyphosate	-38,6398	-38,0904	-37,5901	0
α_{NG}	nitrogen*glyphosate	0,383393	0,38852	0,394205	1
γ_0	control	171,4153	171,8321	172,249	1
γ_N	nitrogen	-0,04382	0,091989	0,216769	0,818239
γ_G	glyphosate	20,92615	21,46587	21,9256	1
γ_{NG}	nitrogen*glyphosate	-0,20752	-0,10133	0,032591	0,094914

TABLE 3.3.3.
MARGINAL POSTERIOR DISTRIBUTIONS OF THE PARAMETERS K, A, AND Γ OF THE GOMPETZ CURVES FOR FLOWERING OF *TANACETUM VULGARE*. THE PARAMETERS IN THE GOMPERTZ GROWTH MODEL, K, A, AND Γ, ARE GENERALIZED AS SHOWN IN EQN. (2), WHERE THE SUBSCRIPT 0 DENOTES THE VALUE OF THE GROWTH PARAMETER IN THE UNTREATED CONTROL, THE SUBSCRIPT *N* DENOTES THE EFFECT OF NITROGEN, *G* THE EFFECT OF GLYPHOSATE, AND *N*G* THE INTERACTION EFFECT. ALL PARAMETERS WERE SIGNIFICANT EXCEPT FOR K_{NG} , Γ_N AND Γ_{NG}

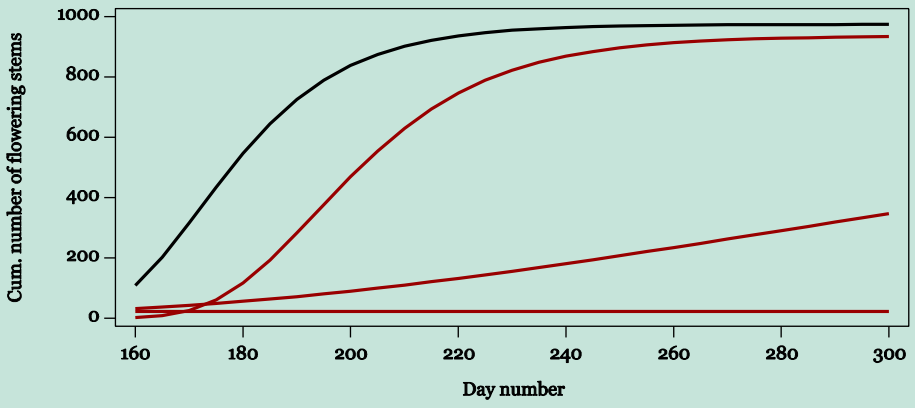

FIGURE 3.3.4.
GOMPERTZ GROWTH FLOWERING CURVES OF *LEUCANTHEMUM VULGARE* FOR GLYPHOSATE TREATMENTS WITH NO NITROGEN ADDED. BLACK CURVE: UNTREATED CONTROL, RED CURVES (FROM TOP): APPLICATION OF 1%, 5%, 25% GLYPHOSATE. FLOWERING CURVES INCLUDE PARAMETER UNCERTAINTY (LINES ARE SHOWN WITH 95% CREDIBLE INTERVALS). NOTE THAT THE UNCERTAINTY IS VERY SMALL.

Due to scarcity of flowers, only data for glyphosate treated plots (1%, 5% and 25%) and untreated controls (0%) were included in the analyses of flower size. In the mixed model analysis with plant individuals within blocks as random effects and treatment as fixed effects, both flower disk diameters and ray petal lengths decreased with glyphosate application (Disks: estimate = -

0.205912, SE = 0.05329285, df = 286, t = -3.86378, *P* = 0.0001, Ray petals: estimate = -0.488894, SE = 0.05766406, df = 286, t = -8.47832, P < 0.001). However, variance of random effects highly exceeded the treatment effects (86% and 85% of variance in disk diameter and ray petal length, respectively, explained by plant individuals).

Only few flower-visiting insect species were observed on flowers of *Leucanthemum vulgare* during the pollinator observation trials. Thus, pollinator diversity and abundances were not compared among treatments. Flower-visitors encompassed a wide range of taxonomic groups (Table 3.3.4).

Order	Families	Species
Coleoptera	Cerambycidae, Chrysomelidae, Scarabaeidae,	*Stenurella melanura, Phyllopertha horticola*
Diptera	Empididae, Muscidae, Syrphidae	*Eristalis lineata*, *Helophilus pendulus*, *H. trivittatus, Sericomyia silentis, Sphaerophoria scripta*
Hymenoptera	Halictidae, Colletidae	*Sphecodes* sp., *Hylaeus* sp.
Lepidoptera	Hesperiidae	*Ochlodes sylvanus*

TABLE 3.3.4.
INSECTS OBSERVED VISITING FLOWERS OF *LEUCANTHEMUM VULGARE*

3.4 Effects of nitrogen and glyphosate on soil fauna

3.4.1 Effects of the treatments on earthworms

Six species of earthworms were found in the 120 soil blocks thoroughly searched by hand sorting. The abundance and g fresh weight (F.W.) of these six earthworms were enumerated from in the soil blocks. The blocks contained relatively few earthworms both in terms of abundance and biomass (Table 3.4.1.1). This result is not surprising given the sandy soil type. Worms of the epigeic life-form were dominating together with one endogeic species, *A. tuberculata*. *L. rubellus* was very rare and only one adult *L. terrestris*[1] were found, but we found a large number of juvenile *Lumbricus spp.* that could not be identified to species. It seems that anecic life-forms were rare, transient and not well established in the habitat, but this has to be confirmed by identification of the juveniles of the genus *Lumbricus*, possibly by barcoding. However, it is most likely, that they would be juvenile *L. rubellus* due to the presence of leaf litter, which is their preferred habitat.

The two dominant species, earthworms juvenile *D. octaedra* and *A. tuberculata*, were both affected by the nitrogen fertilisation, but in opposite manners. *D. octaedra* were stimulated by the N fertiliser and *A. tuberculata* were inhibited at the highest N application rate compared to the other rates (Fig. 3.4.1.2. and 3.4.1.3).

[1] COI barcoding suggests that this is actually *Lumbricus herculeus* and the juvenile *Lumbricus* are also *L. herculeus*.

Species	Numbers	F.W., g	Life-form (composite trait)
Juvenile *Lumbricus* sp.	68	24.9	Epianecic
Dendrobaena octaedra	107	11.7	Epigeic
Aporrectodea tuberculata	24	11.2	Endogeic
Dendrodrilus rubidus	23	3.4	Epigeic
Lumbricus terrestris	1	2.4	Anecic
Lumbricus rubellus	1	0.7	Epigeic
Aporrectodea longa	1	0.1	Endoanecic
Total worms recorded	225	54.4	
Total mean earthworm abundance m^{-2} – across treatments	41	6.9	
Total mean earthworm abundance m^{-2} – controls	24	6.0	

TABLE 3.4.1.1.
EARTHWORM SPECIES RECORDED AND THEIR ABUNDANCE AND G F.W. IN THE COMPLETE SET OF SAMPLES COLLECTED OCTOBER 2012, AS WELL PER SQUARE-METER (M^{-2}) FOR TOTALS.

Species	Effect	F Value	Pr > F
		.	.
***A. tuberculata*, F.W. g m^{-2}**	N	5.54	0.5%
***A. tuberculata*, indv. m^{-2}**	N	4.9	0.9%
***Dendrodrilus rubidus* indv. m^{-2}**	N	3.64	3.0%
***Dendrobaena octaedra* F.W. g m^{-2}**	N	4.7	1.1%
***Dendrobaena octaedra* F.W. g m^{-2}**	N*Gly	2.91	1.2%
***Dendrobaena octaedra*, indv. m^{-2}**	N*Gly	2.99	1.0%

TABLE 3.4.1.2.
OUTCOME OF A MIXED MODEL ANALYSIS OF TRANSFORMED EARTHWORM ABUNDANCE AND BIOMASS. THE TABLE INCLUDES ONLY THE SPECIES THAT WERE SIGNIFICANTLY AFFECTED BY THE MAIN FACTORS AND THEIR INTERACTIONS.

D. rubidus had a higher abundance at the 25 kg N y^{-1} ha^{-1} compared to the two other N application rates (P<5%, Tukey's test, Fig. 3.4.1.3). So, in fact, we observed four different patterns of responses to the fertilisation when including the *Lumbricus* sp. as indifferent or with an undetectable response.

There were also effects of the glyphosate application, though only detectable for *D. octraedra*, but they differed between levels of nitrogen applications rate, as reflected in the significant interaction between these two factors (Table 3.4.1.2). So, at zero level of fertilization *D. octraedra* was stimulated by increasing levels of glyphosate, this was probably opposite for juv. *Lumbricus* sp. and a regression of abundance and glyphosate for this species with no fertilisation supports this pattern.

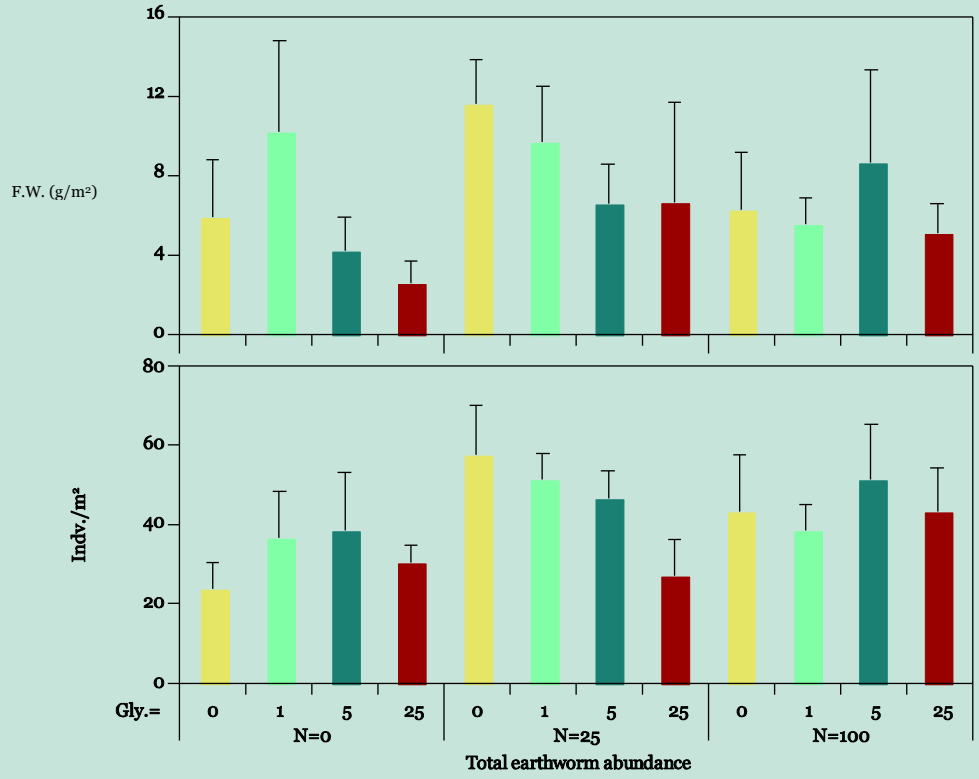

FIGURE 3.4.1.1
ABUNDANCE . INDV. WORMS M^{-2} AND BIOMASS, g F.W. M^{-2} OF TOTAL EARTHWORMS FOR ALL TREATMENT COMBINATIONS. LOWER ROW OF X-AXIS VALUES, 0, 25 AND 100 IS APPLICATION RATE OF N KG Y^{-1} HA^{-1}. UPPER ROW OF X-AXIS VALUES IS THE APPLICATION RATE OF GLYPHOSATE IN PERCENTAGE OF THE RECOMMENDED APPLICATION RATE OF 360 G. A.I. HA^{-1}.

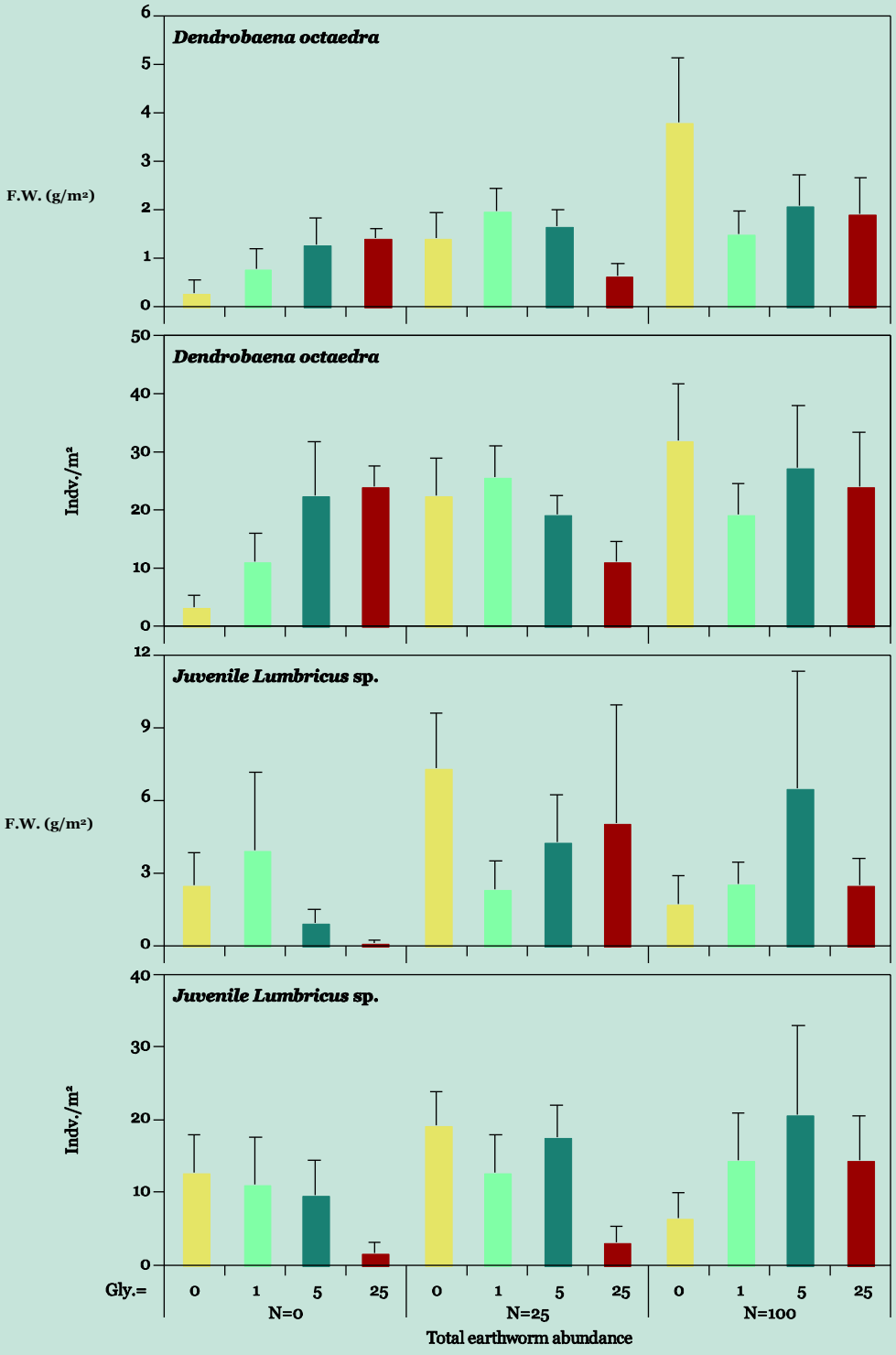

FIGURE 3.4.1.2.
ABUNDANCE AND BIOMASS M^{-2} OF *D. OCTRAEDRA* AND JUVENILE *LUMBRICUS* FOR ALL TREATMENT COMBINATIONS.

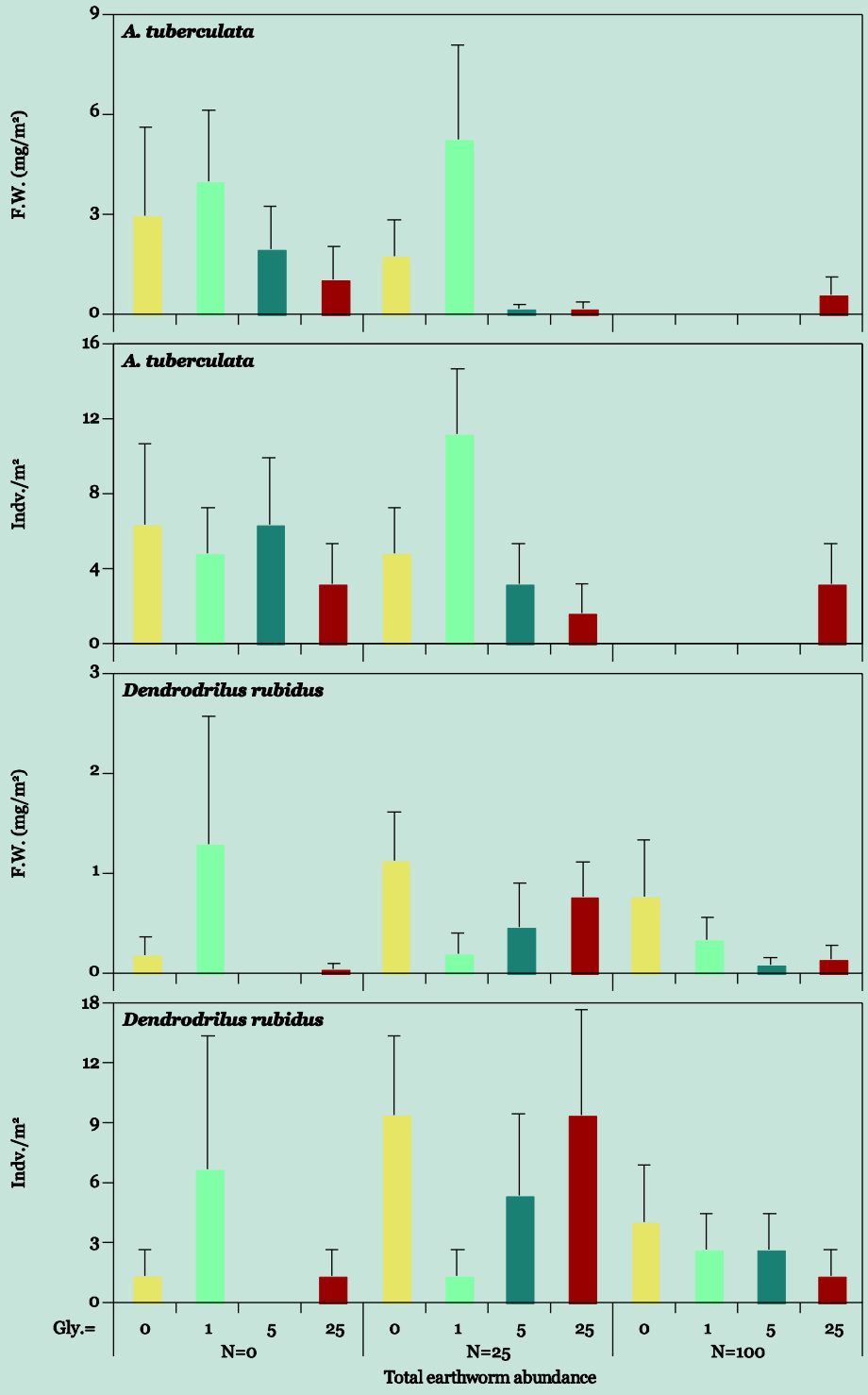

FIGURE 3.4.1.3.
ABUNDANCE AND BIOMASS M^{-2} OF *A. TUBERCULATA* AND *D. RUBIDUS* FOR ALL TREATMENT COMBINATIONS.

3.4.2 Earthworm traits

To extend the earthworm traits as included in Appendix 2, we added an additional trait which we named "trophic position". This trait is based on the isotopic signatures $\delta^{13}C$ and $\delta^{15}N$, which were converted into a combined trait scaled to the range of 0 to 1. In the first step done to calculate this index, the mean signatures were estimated from the data for the individual species and stages for each levels of N fertilization (Fig. 3.4.2.1). Then a linear regression of $\delta^{15}N$ on $\delta^{13}C$ was made to map the two variables into one dimension and the projection of $\delta^{15}N$ upon this dimension was used as a trophic position index. As shown in Fig. 3.4.2.1, *L. rubellus* did not fall into the group of litter-feeding anecics and epigeics, but was grouped with *A. tuberculata,* contrary to early findings by (Schmidt et al. 1997, Neilson et al. 2000, Briones and Bol 2003). Nevertheless, it has been termed epi-endogeic because it mixes organic matter into the top soil, creating soil aggregates with incorporated root and leaf material (Addison 2009, Sánchez-de León et al. 2014). However, as only one individual was found, it will not affect the trait-based analyses and should only be noticed as a particular habitat-dependent special case of trophic position, as none of the cited studies covered a similar dry nutrient poor sandy habitat. A similar case was observed for *A. longa,* which turned out to be purely soil feeding, while it expectedly should be endo-anecic with a signature between the epigeics and the endogeics. Again, it will not affect the analytical outcome, as only one individual was found. The soil feeding trophic position by *D. rubidus* may seem more controversial, as this species is considered to be strictly epigeic and, therefore, primarily a litter feeder. However, scrutinizing the literature for isotopic composition values, confirms that it could very well be considered to be epi-endogeic, as it was found to have an intermediate $\delta^{15}N$ compared to endogeics and epigeics and anecics in deciduous woodland (Neilson et al. 2000).

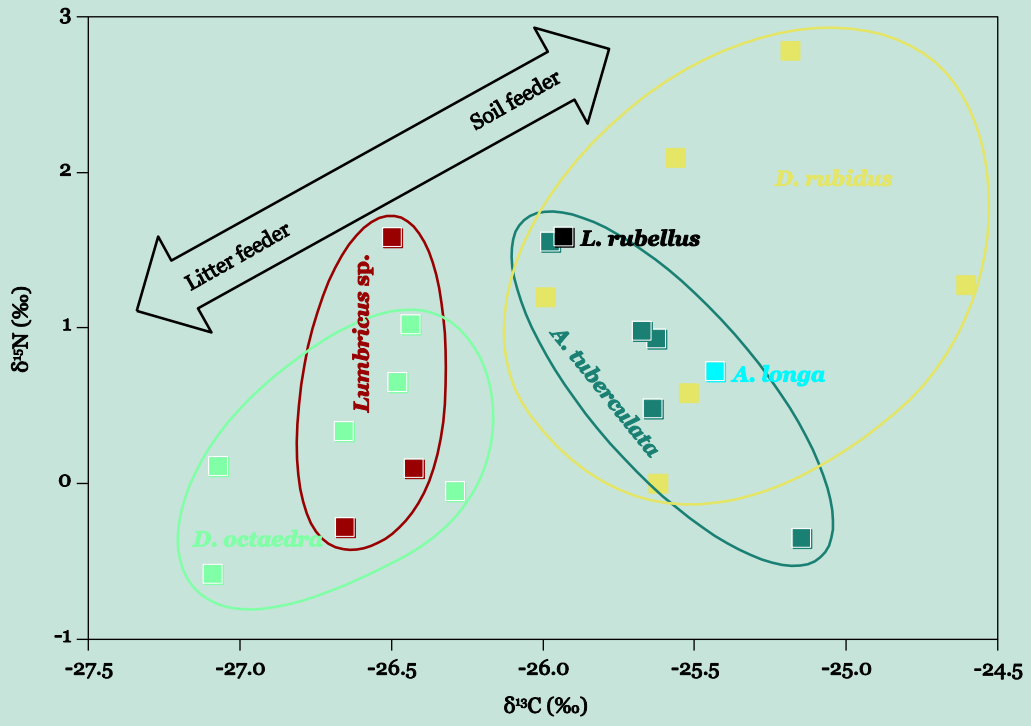

FIGURE 3.4.2.1.
MEAN ISOTOPIC SIGNATURES, $\delta^{13}C$ AND $\delta^{15}N$, OF JUVENILE AND ADULT EARTHWORMS COLLECTED FROM THE FIELD LOCATION ON OCTOBER, 2012.

3.4.3 Trends in microarthropod population responses

We found an average of 440 [410-460] 10^3 microarthropods m^{-2} dominated by 330 [310-350] 10^3 mites m^{-2} over the 110 [100-120] 10^3 collembolans m^{-2}. No common unidirectional responses were observed for microarthropods, hence, the sum of all species and groups showed no significant effects (Fig. 3.4.3.1 Microarthropods, Table 3.4.3.1). There was no common pattern among microarthropods in the response to the treatments. Collembolans were stimulated by the N fertilization, except for the sminthurids Sminthurides sp.(data not shown) and *Sminthurinus elegans*. Hence, Folsomia fimetaria increased from being rare at only 240 indv. m-2 [0-500] to 4.2 [2.5-6.0] 103 m-2, a 17-fold increase (Fig. 3.4.3.5). *P. notabilis* was also stimulated by the fertilization, but simultaneously irregularly stimulated by the herbicide (Fig. 3.4.3.2); it was doubling its abundance from 14 [11-18] 103 m-2 in the control N to 29 [21-36] 103 m-2 at 100 kg N ha-2. *I. viridis* (Fig. 3.4.3.4) significantly increased its numbers with increasing glyphosate application rate only at the 25 kg ha-1, while *M. anurida* followed an opposite trend of decreasing population abundance with increasing glyphosate (Fig. 3.4.3.4). Glyphosate had only negative effects on mites, while for Gamasida (Fig. 3.4.3.4), Galumnoidea and *P. peltifer* (Fig. 3.4.3.5) it differed for the levels of N, as indicated by the significant interaction between N and glyphosate (Table 3.4.3.1).

3.4.4 Collembolan trait based analyses of treatment effects

Collembolan trait scores were subject to the same mixed model analysis as the population abundances. A number of traits were responding to the treatments, none of which lent itself to an easy interpretation (Table 3.4.3.2). The moisture preference was especially affected at 25 kg N ha^{-1}.

	N	Glyphosat	N*Gly
Collembola	*↗	*↗	
Acari	*↘		
Actinedida	*↘		
Oribatida		*↘	
Tullbergiinae		*↗	
Oppioidea		*↘	
Parisotoma notabilis			*
Cryptopygus scapellifer			*
Uropodina		*↘	
Sphaeridia sp.	*↘		
Tarsonemidae	*↗		
Astigmata	*↗	*↘	
Gamasida*			*
Micranurida pygmaea			*
Folsomia fimetaria	*↗		
Sminthurinus elegans	*↘		
Galumnoidea			*
Platynothrus pelitifer		*↘	*
Neanura muscorum	*↗		

*EXCL. THE UROPODINA AND THE RHODACARIDAE.

TABLE 3.4.3.1.

MICROARTHROPOD SPECIES OR GROUPS WITH A SIGNIFICANT OUTCOME OF EFFECTS OF FACTORS IN A MIXED MODEL ANALYSIS: NITROGEN FERTILIZATION, N, AND GLYPHOSATE, GLY, AND THEIR INTERACTION, N□GLY.
*: SIGNIFICANT F VALUE P<5%. ↗ STIMULATING TREND OR ↘ DECREASING TREND.

	N	Glyphosat	N∗Gly
Body pigmentation pattern	*		*
Furca development			*
Antenna estimated length			*
Life form sensu Gisin (1943, 1947, 1948)		*	
Life form (Rusek 1989)			*
Moisture preference		*	
Habitat width			*
Mode of reproduction			*
Mouthparts		*	*

TABLE 3.4.3.2.
COLLEMBOLAN TRAIT SCORES WITH A SIGNIFICANT OUTCOME OF EFFECTS OF FACTORS IN A MIXED MODEL ANALYSIS: NITROGEN FERTILIZATION, N, AND GLYPHOSATE, GLY, AND THEIR INTERACTION, N∗GLY. *: SIGNIFICANT F VALUE P<5%.

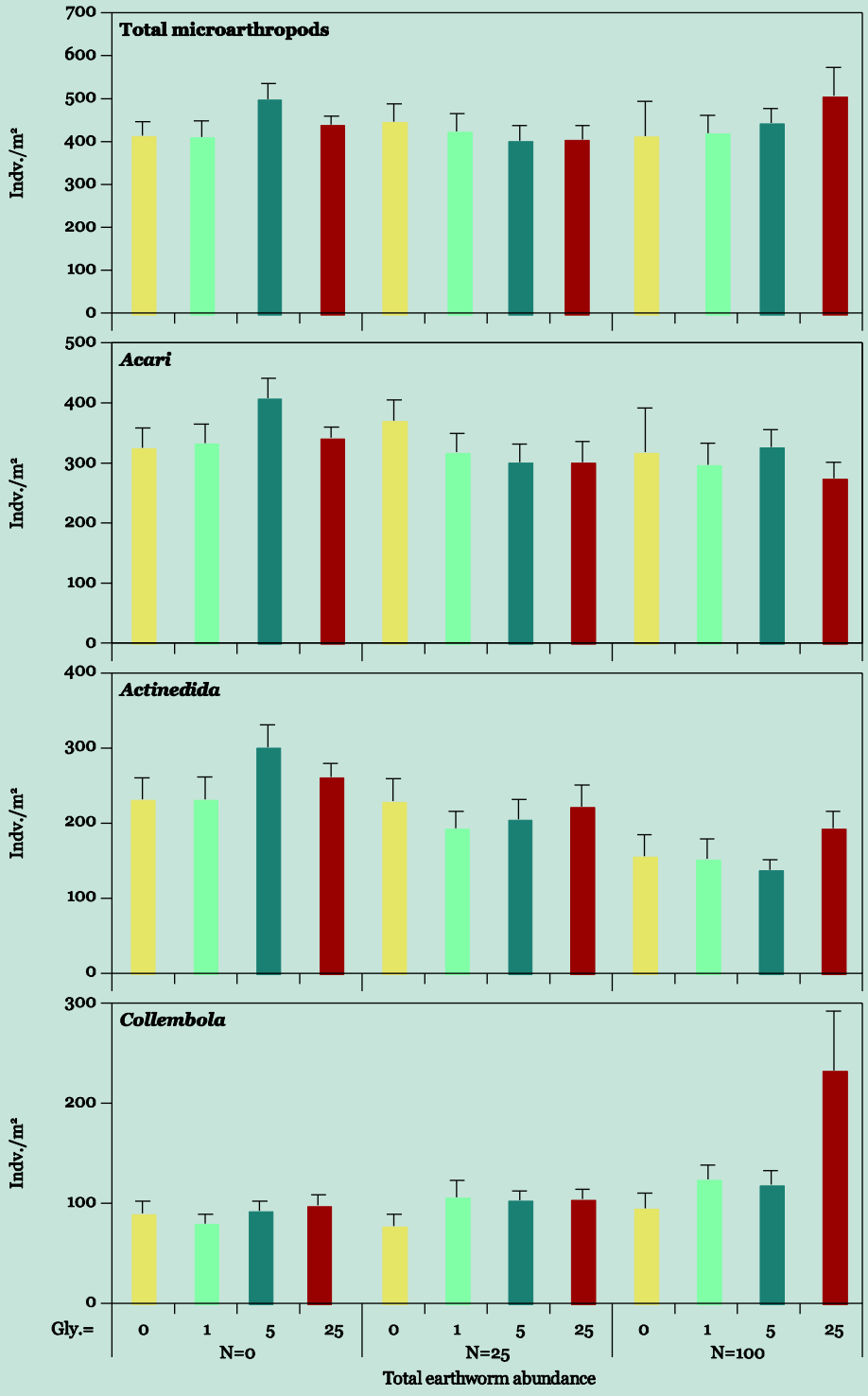

FIGURE 3.4.3.1.
MEAN ABUNDANCE IN 1000 INDV. M^{-2} FOR MICROARTHROPOD SPECIES. VERTICAL LINES ON BARS ARE 1 STANDARD ERROR OF THE MEAN. GLYPHOSATE TREATMENT, GLYPH., IN THE DOSES 1, 5, 25% OF RECOMMENDED FIELD APPLICATION; N IS THE ANNUAL N APPLICATION RATE: 25 AND 100 KG HA^{-1}. 0 IS THE UNTREATED SOIL.

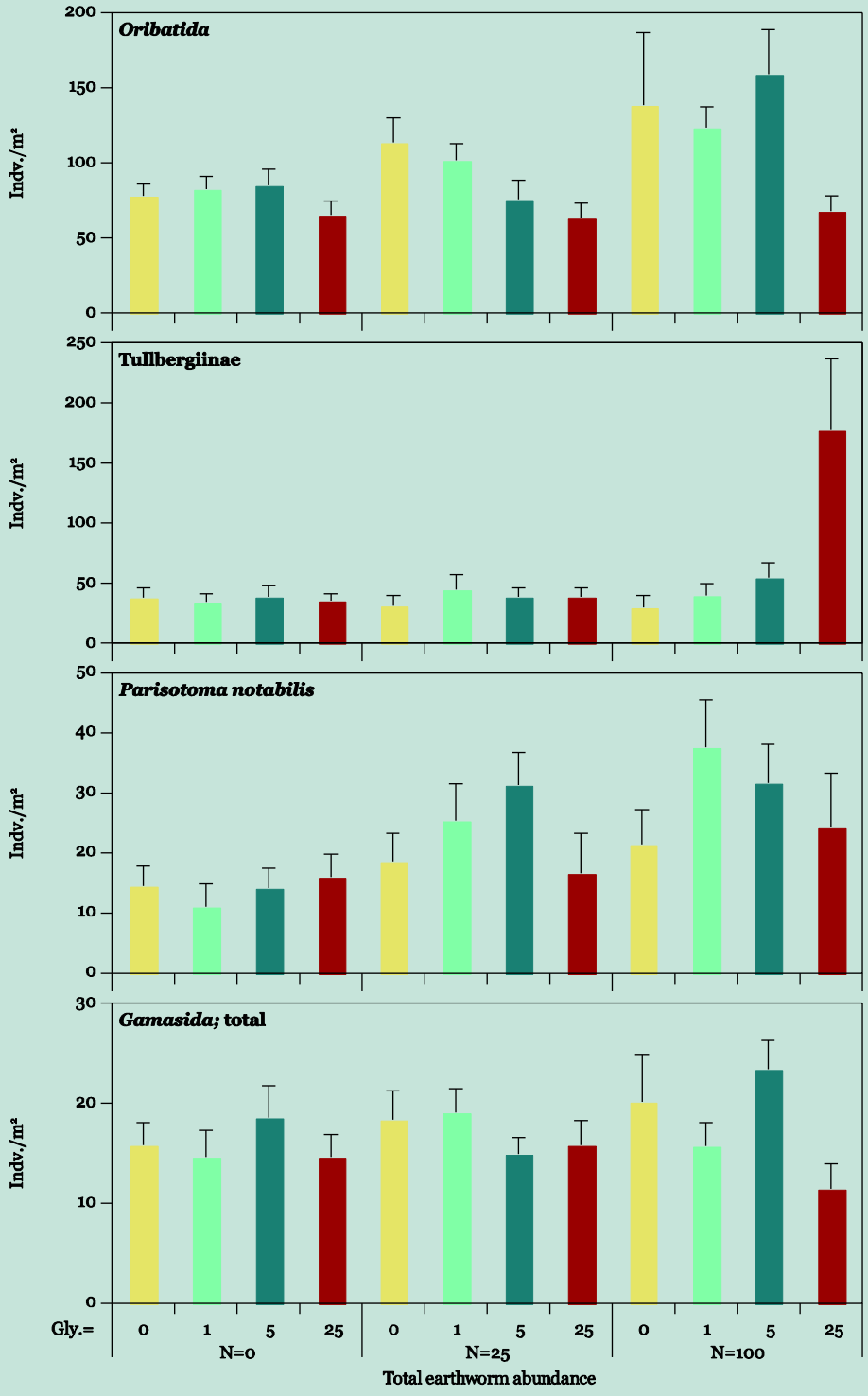

FIGURE3.4.3.2.
MEAN ABUNDANCE IN 10^3 INDV. M^{-2} FOR MICROARTHOPOD SPECIES.

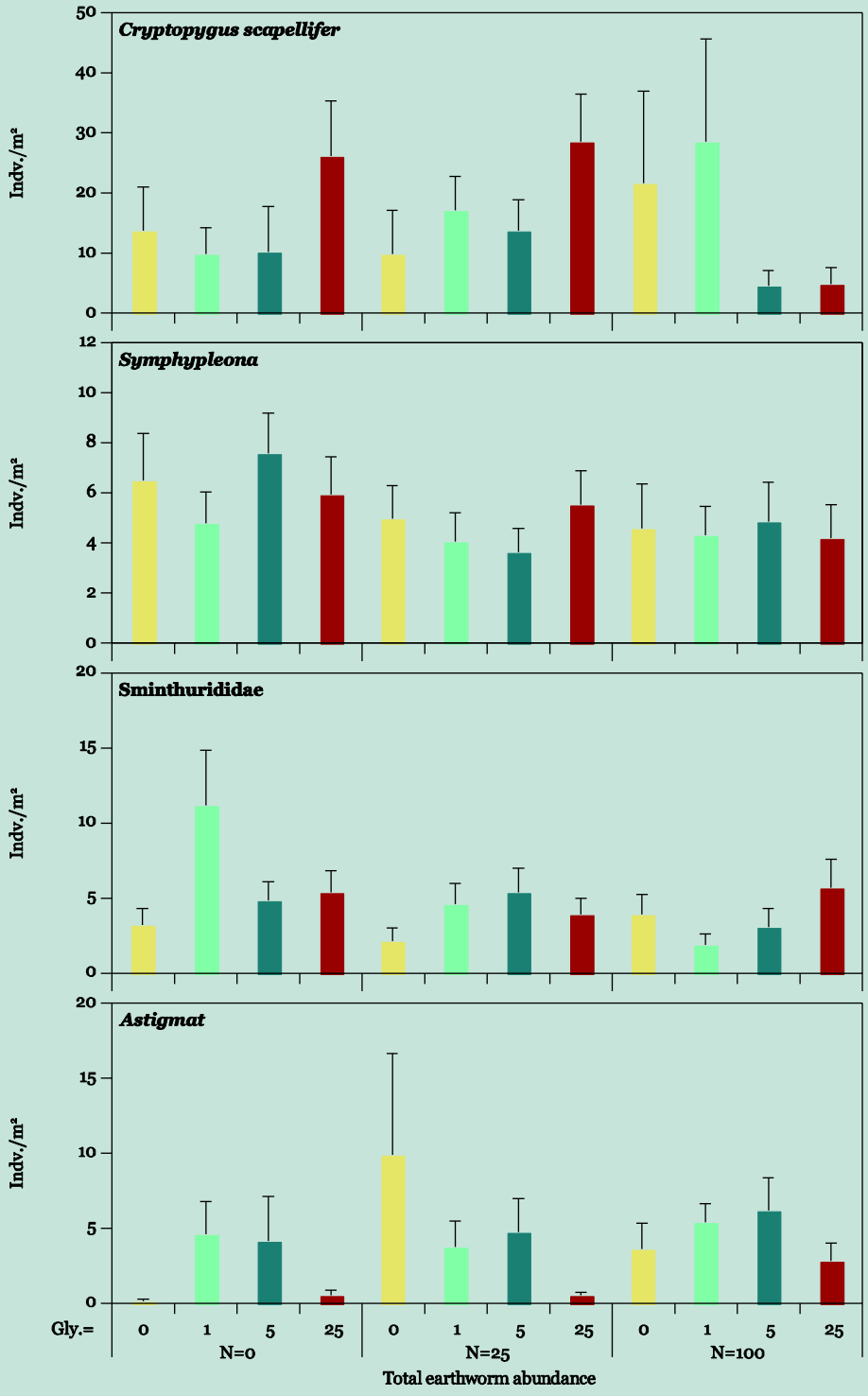

FIGURE 3.4.3.3.
MEAN ABUNDANCE IN 1000 INDV. M^{-2} FOR MICROARTHOPOD SPECIES.

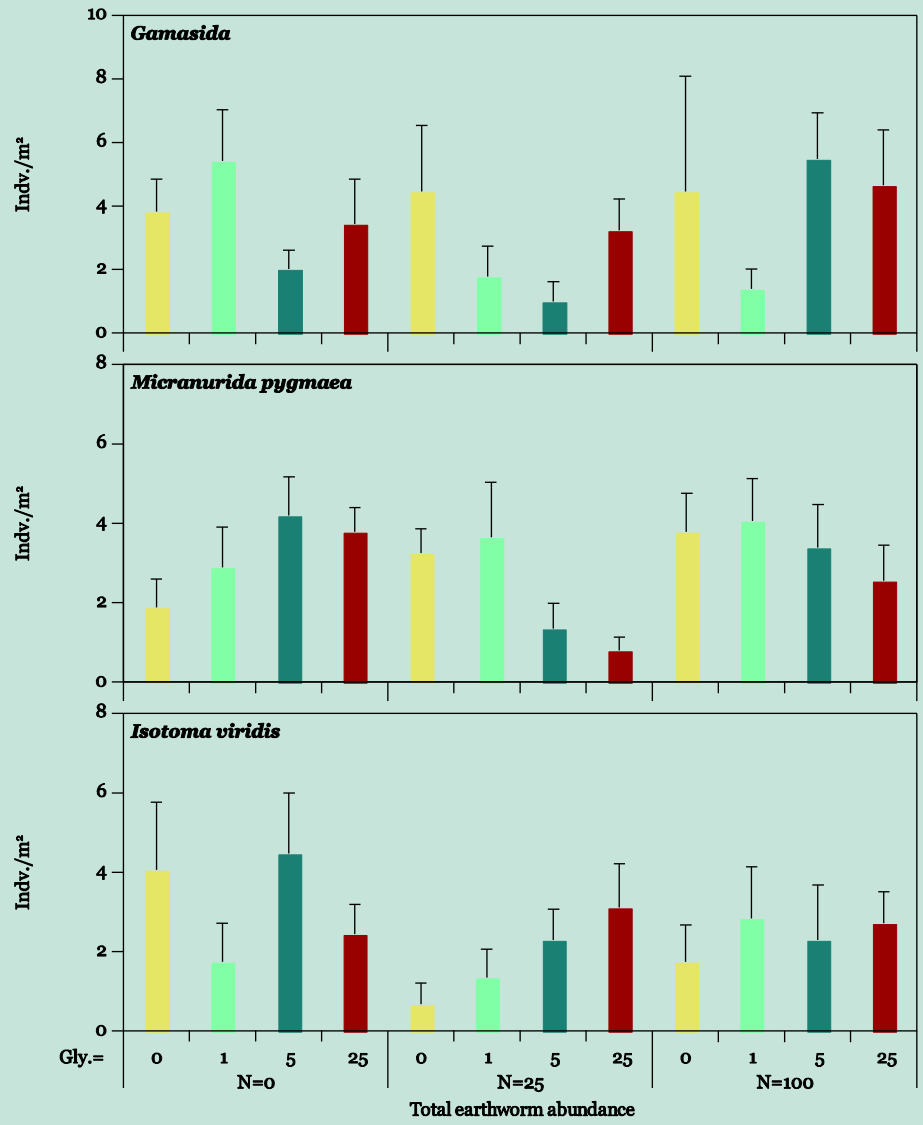

FIGURE 3.4.3.4.
MEAN ABUNDANCE IN 1000 INDV. M^{-2} FOR MICROARTHROPOD SPECIES. THE GAMASIDA GROUP DID NOT INCLUDE THE UROPODINA AND THE RHODACARIDAE.

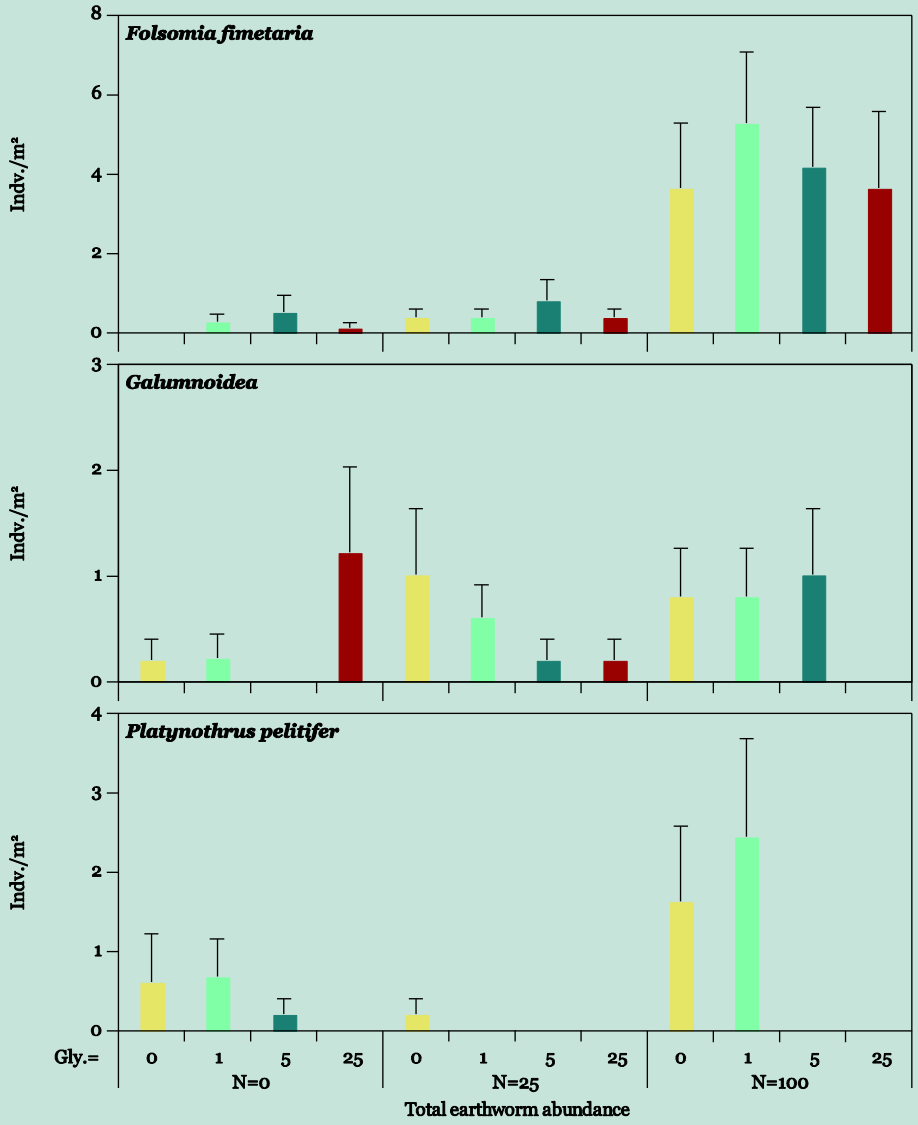

FIGURE 3.4.3.5.
MEAN ABUNDANCE IN 1000 INDV. M^{-2} FOR MICROARTHOPOD SPECIES.

3.5 Stable isotopes and C:N

3.5.1 General trophic structure and food-web positions

The trophic grouping according to the isotopic signatures is shown in Fig. 3.5.2.1, which is a compilation of all isotopic data across all treatments. The predatory mite, Gamasida, forms a cluster with the highest observed $\delta^{15}N$ of 5.5 ‰, in agreement with their consumption of detrivores such as collembolans. As neanurid collembolans are known to be predators, this is confirmed by the position of *N. muscorum*, mean $\delta^{15}N$ of 4 ‰. Litter feeding earthworms (Fig. 3.4.2.1) and detritivourous collembolans tend to fall into the same cluster, which is the left part of the earthworm cluster. On average, the position of litter, the main food of detritivores, is 2Δ‰ below earthworms and collembolans, while excluding the *Isotoma* sp. ($\delta^{15}N$ -2.3 ‰) that seems to feed on living plant or algae sources, which we did not include in our inventory. The collembolans *Lepidocyrtus* sp., Onychiuridae, *Folsomia* sp. and Tullbergiinae formed a group with a mean $\delta^{15}N$ of 1.6 ‰ in contrast to *P. notabilis* with a mean $\delta^{15}N$ of 0.2 ‰.

We included 29 aphids as a reference to compare the detritivores with a true herbivore. Aphids turned out to have the lowest observed $\delta^{13}C$ of -30 ‰, 1 Δ‰ lower than the lowest observation for *Isotoma* sp. of -29‰. This difference could indicate a tropic distance of one level, but most likely indicates that this collembolan does not acquire its carbon from fresh plant material similar to the aphid carbon source.

The signature of the humus or soil organic matter represented by the soil cluster in Fig. 3.5.2.1 is situated above the detrivorous collembolans and earthworms and, consequently, could not form a significant proportion of their diet. This is not surprising, as humus by definition is recalcitrant and inaccessible to decomposition and digestion.

3.5.2 Influence of the treatments on the trophic structure

The N fertilizer applied in spring 2012 contributed a $\delta^{15}N$ of -2.5 ‰ to the plant and soil system. The $\delta^{15}N$ signature of soil organic matter or humus was unaffected by the level of N application with its average $\delta^{15}N$ of 2.0 ‰ [1.7–2.2] across the range of N application rates including the controls. Changing the $\delta^{15}N$ signature of humus may require decades, i.e. incorporation of fertilizer N is a slow process.

Isotopic signatures of shoots and their attached roots were established for the species *F. ovine*, *A. capillaris*, *H. pilosella*, *E. repens*, *L. vulgare*, *T. vulgare*, *A. millefolium*, *E. esula*. Overall roots were enriched in $\delta^{13}C$ by 1.1 ‰ compared to shoots, but very much depending on species ranging between zero and three ‰.

The N fertilization created a significantly lower δ15N signature for plants, decreasing by 1‰ [0.6 – 1.5] at both 25 and 100 kg N ha-1 compared to the unfertilized control (Fig. 3.4.2.1). Only δ15N of L. vulgare did not respond to N fertilization and, therefore, did not take up fertilizer derived nitrogen. Although the δ13C was increased by 1.2 ‰ from the control to 25 Gly, this was not proven significant. Otherwise, C:N of these plant species did not differ and were not affected by the treatments.

The signatures of litter reveal only minor variability and no influence by the Gly treatment. After removal of the block effect and the interaction between the block and treatment effect by the mixed model, there were no effects of Gly, neither on $\delta^{15}N$ nor on $\delta^{13}C$. In the 100 N treatment, $\delta^{15}N$ was significantly enriched by 0.6‰, P<1%, indicating that this was not due to an inorganic fertilizer contribution to N content in the plants, as this should have led to a depletion of ^{15}N. Likewise, in the 25 Gly treatment $\delta^{13}C$ was statistically only slightly increased (P=7%) by 0.9 [-0.1 – 1.9].

Litter quality was significantly higher in the 100 N treatment, P<1‰, with a C:N of 22 in 100 N compared to an average C:N of 40 in the control and 25 N treatments. This was due to an increase in litter total N % content by 50% at 100 N compared to the other two levels (P<1%) as well as a decrease in C content by 15%, although not statistically significant, but still contributing to the increased litter quality.

Largely, the $\delta^{15}N$ signature of microarthropods did not decrease with the increase in N. On the contrary, of the eight collembolans for which a comparison between levels of N was possible, we found five species with significantly increased $\delta^{15}N$ signature with increasing N application rate. This indicates that those collembolans did not derive their food from sources directly dependent on the N fertilizer. However, the tullbergid collembolans decreased their $\delta^{15}N$ signature from 2.8 ‰ [1.4 – 4.1] at 0 N to -0.9 [-1.9 – 0.1] at 100 N, conversely indicating that they derived their food from sources directly dependent on the N fertilizer.

Microarthropod $\delta^{13}C$ increased by 1.0 [0.5 –1.5], P<1‰ in 25 Gly compared to the joint 0, 1 and 5% of the normal glyphosate rate. In particular, *N. muscorum* and the Onychiuroidea, comprising Tullbergiidae and Onychiuridae, seem to contribute to this enrichment.

Earthworm $\delta^{15}N$ isotopic signatures (Fig. 3.2.1.1) were not affected by the treatments. The $\delta^{13}C$ increased with increasing glyphosate by an average of 0.7 ‰ [0.2–1.3] (P<1%), which was contributed particularly by *D. octaedra, D. rubidus* and *Lumbricus sp.* and with a minor contribution by *A. tuberculata*.

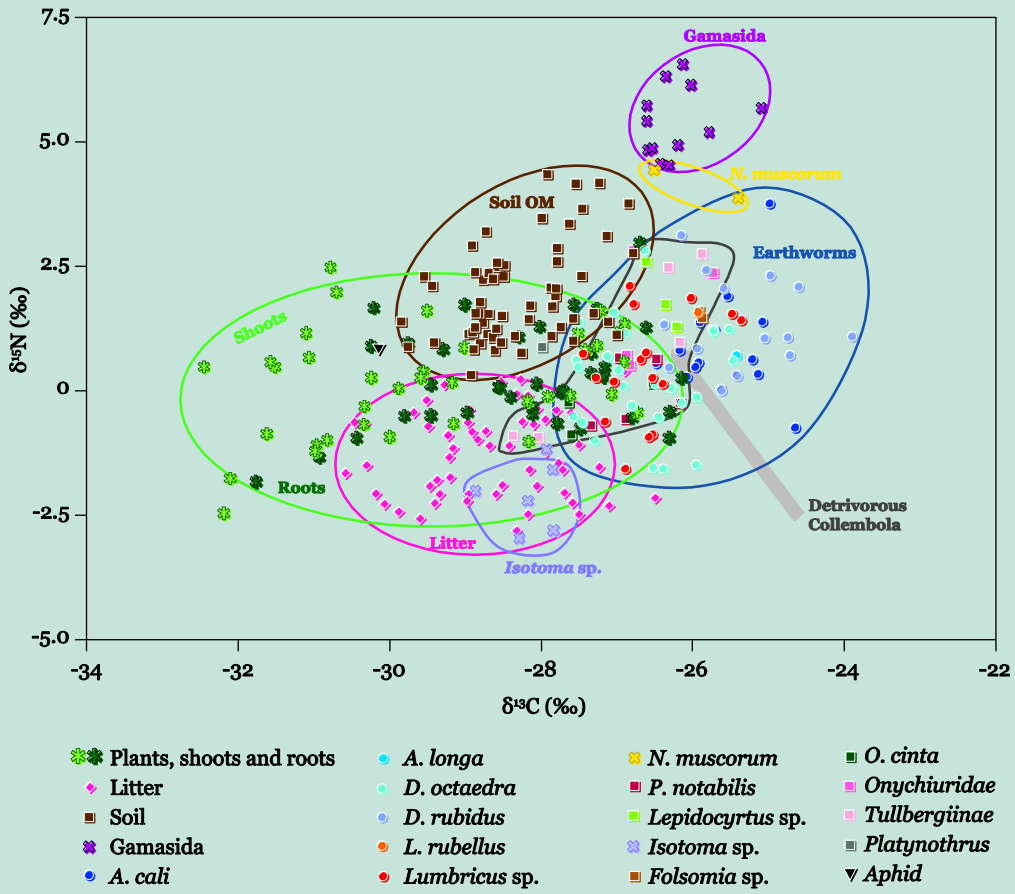

FIGURE 3.5.2.1.
PLOT OF THE ISOTOPIC SIGNATURES $\Delta^{13}C$ AND $\Delta^{15}N$ INCLUDING ALL DATA. CLUSTERS OF SIMILAR TYPES OF MATERIAL AND TAXONOMIC GROUPS ARE OUTLINED. SEE TEXT FOR EXPLANATION AND INTERPRETATION.

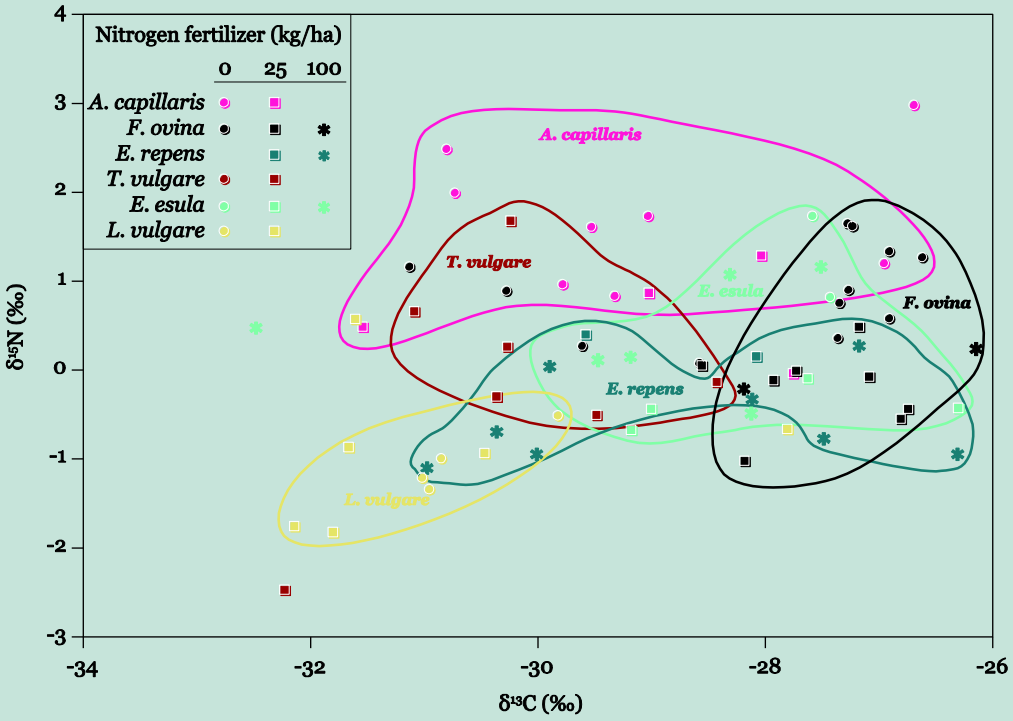

FIGURE 3.5.2.2.
PLOT OF THE ISOTOPIC SIGNATURES $\Delta^{13}C$ AND $\Delta^{15}N$ FOR THE PLANT SPECIES INDICATING THE TREATMENTS OF NITROGEN FERTILIZER. CLUSTERS OF SPECIES ARE OUTLINED. SEE TEXT FOR EXPLANATION AND INTERPRETATION.

3.6 Effects of nitrogen and glyphosate on ecosystem function and properties

3.6.1 Hypothesis on the studied causal relationships

The effect of nitrogen and glyphosate on ecosystem function and properties was investigated using structural equation models (SEM) where the hypothesized causal relationships are motivated below.

Plants

Both nitrogen and glyphosate as well as the interaction of the two drivers have been shown to exert strong selection pressures on the plant community, including affecting both the competitive interactions and plant demographic parameters (Holst 2008, Damgaard et al. 2011, Strandberg et al. 2012, Damgaard et al. 2013, Loïc et al. 2014, Damgaard et al. submitted-b). In the present structural equation modelling, we are primarily interested in the indirect effect of nitrogen and glyphosate on the soil fauna that is mediated by the altered plant community. We hypothesized that the specific niche requirements and food availability of the soil fauna may be modelled by i) the turnover rate of plants (modelled by the proportion of annual plants), ii) root morphological characteristics (modelled by the proportion of dicots) iii) root biomass measured at two soil depth, iv) leaf economy and litter turnover rate (modelled by the plant traits SLA and LDMC, respectively (Fortunel et al. 2009), which *a priori* were assumed to be correlated).

Earthworms

The experimental site has been without soil tillage for >10 years, nor have plant residues been removed by harvest or other means. Such conditions are normally beneficial for earthworms, promoting high abundance and biomass because food conditions are good and physical disturbance is not limiting survival or growth (Edwards and Bohlen 1996). Therefore, competition for food is

hardly a reason for the low abundance of earthworms at the site. Rather, it is likely that the sandy texture of the soil and, consequently, a poor water retention capacity is the main limiting factor for earthworms at the site. High plant biomass can influence soil water potential by increasing the litter layer that would reduce evaporation of water from the soil surface. However, growing plants remove water from the entire root zone by their evapotranspiration. This process is much more efficient than removal of soil water by evaporation from the soil surface (Hillel 1998). Based on these considerations, we hypothesize that earthworm abundance and biomass (especially of endogeic species) are negatively correlated with plant biomass in the ecosystem. Earthworms are highly sensitive to even moderate reductions of soil water potential (Holmstrup 2001). Consequently, increased N-input will reduce earthworms due to higher plant biomass and more frequent occurrence of stressful drought conditions. Direct toxic effects of glyphosate in lower than recommended dosages are unlikely to occur (Springett 1992, Dalby et al. 1995a), but glyphosate will reduce plant biomass and, consequently, earthworms will be stimulated according to the same considerations as for nitrogen addition.

Microarthropods: Collembola and Acari
Collembola and Acari are much less sensitive to low soil water potential than are earthworms (Holmstrup and Bayley 2013, Holmstrup et al. 2013), which suggests that soil water relationships are unlikely to be of importance for these groups. Springtails and oribatids are important grazers of fungi and contribute to fragmentation of dead plant material, thereby enhancing decomposition processes in the soil (Faber, 1991; Seastedt, 1984; Verhoef and Brussard, 1990; Bardgett 2005; Ngosong et al. 2011). Further, predacious soil mites exert top-down control of secondary decomposer organisms, which can also influence decomposition rates (Hedlund and Öhrn, 2000; Karg, 1961; Koehler, 1997). A higher plant biomass will likely increase root exudation of labile carbohydrates, the amount of plant litter and the microbial decomposers of dead plant tissue, and this is likely to stimulate all microarthropod groups and, in particular, epedaphic Collembola, which depend on a rich litter layer as a suitable habitat. We therefore hypothesize that abundance of microarthropods will increase in N-fertilized plots through increased plant productivity that will deliver increasd food supply for both soil dwelling and surface dwelling species of microarthropods. Glyphosate applied in low doses are unlikely to have direct toxic effects on microarthropods (Eijsackers 1985). We therefore hypothesize that glyphosate will decrease abundance of microarthropods due to lower plant productivity and therefore lower food supply. Our third hypothesis concerning microarthropods is that effects of N-fertilization and glyphosate will be additive.

3.6.2 Results of the structural equation modelling

The hypothesized SEM and the fit of the SEM are shown in Appendix 3 and Fig. 3.6.1. The SEM was fitted either i) by simulating the missing values using the lavann procedure: missing="ml", ii) or deleting all records with missing values. Since the results of the SEM fitting approaches were qualitatively similar (results not shown) and the missing values were almost all due to missing root biomass data, we have chosen only to report from the SEM that used all 120 plots in the following. As expected, considering the number of hypothesized causal relationships, the resulting SEM failed to meet the requirement of chi-squared test ($P < 0.001$), although the standardised root mean square residual was 0.095 (Bentler 2007). In this case, some workers would proceed to a model selection procedure by reducing the number of assumed causal relationships until the SEM meet the requirement of chi-squared test. However, we think that such a model selection procedure would result in a rather arbitrary final SEM model, given the number of possible causal relationships and the indispensable departure from multivariate normality (Hooper et al. 2008). Generally, the variance in plant traits explained by the SEM exceeded the variance in soil fauna traits, and only between 5% and 20% of the variance in soil fauna traits were explained by the SEM (Appendix 3). This was expected, since the primary action of the two exogenous drivers, nitrogen and glyphosate, are targeted towards plant growth, and an exceedingly rich literature documents the effects of both nitrogen and glyphosate on plant communities. Consequently, we will report the

results of the fitted SEM with a cautious note that the variance of many of the variables is only poorly explained by the model (Figure 3.6.2.1).

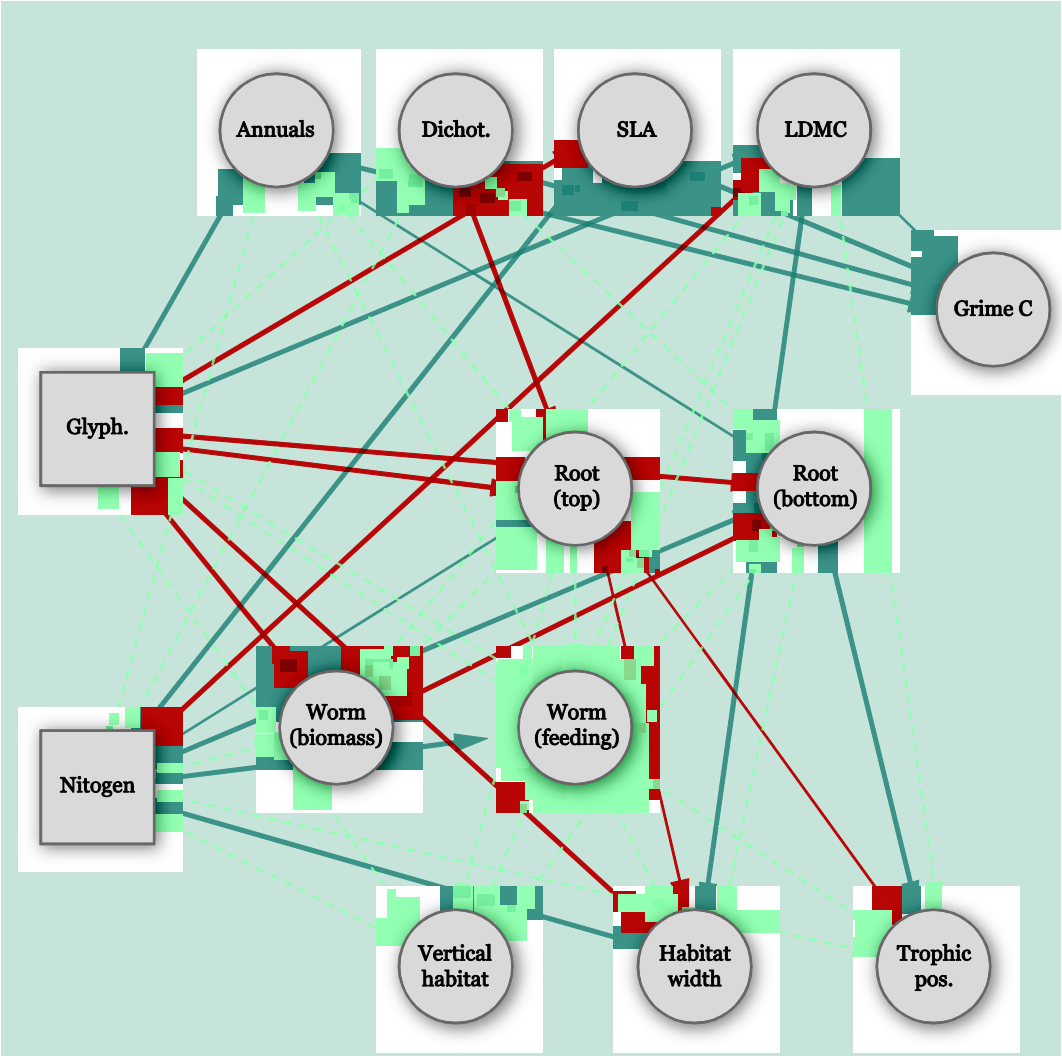

FIGURE 3.6.2.1.
STRUCTURAL EQUATION MODEL OF COMMUNITY WEIGHTED AVERAGE TRAIT VALUE AND OTHER ECOLOGICAL VARIABLES. THE NITROGEN AND GLYPHOSATE TREATMENTS ARE EXOGENOUS VARIABLES. THE FULL MODEL IS SHOWN. RED (BLACK) THICK ARROW IS A SIGNIFICANT NEGATIVE (POSITIVE) EFFECT ($P<0.05$); RED (BLACK) THIN ARROW IS A MARGINALLY SIGNIFICANT NEGATIVE (POSITIVE) EFFECT ($0.05 \leq P < 0.1$); GREY DOTTED ARROW IS A NON-SIGNIFICANT EFFECT ($P \geq 0.1$).

Increasing levels of nitrogen selected for thinner leaves (high SLA, low LDMC), whereas glyphosate oppositely selected for thicker leaves with a higher dry matter content. Glyphosate selected for plant species with an annual life cycle, whereas neither nitrogen nor glyphosate had an effect on the relative proportion of dicots. The plant trait SLA (and LDMC) together with the proportion of dicots were good predictors of the competitive effect of the community, as measured by Grime's C (90 % of the variance in Grime's C was explained by the model).

The root biomass was positively affected by nitrogen and negatively affected by glyphosate. The effect on the roots by the two drivers was mainly caused by direct effects rather than by indirect effects mediated by selection on above ground plant traits. If the plant community was dominated by monocots, then there was a higher root biomass in the top soil.

The SEM analysis indicated that glyphosate had a significant negative effect on total earthworm biomass, although a mixed model ANOVA did not suggest this for any species (Table 3.4.1.2). Nitrogen did not have significant effect on earthworm total biomass, whereas increased nitrogen caused a shift towards earthworms feeding on litter, i.e. species such as *D. octaedra* were stimulated by nitrogen. Root biomass in deep soil layers (Root bottom) had a significant negative influence on total earthworm biomass.

SEM indicated that there was a negative relationship between glyphosate and habitat width of Collembola, whereas nitrogen had the opposite effect. LDMC had a positive effect on habitat width of Collembola. Further, roots in deep soil layers were positively correlated with trophic position of Collembola.

4. Discussion

4.1 Effects of nitrogen and glyphosate on plant species composition

Both fertilizers and herbicides at the concentrations/doses found at the field edge have been documented to affect plant communities of habitats adjacent to the field by reducing species diversity (e.g. Stevens et al. 2004, Clark and Tilman 2008, Kleijn et al. 2009, Strandberg et al. 2012, Pellissier et al. 2014, Schmitz et al. 2014) and changing community composition (Kleijn and Verbeek 2000, Strandberg et al. 2012, Schmitz et al. 2014). The present study support these findings and shows that cover of individual species is affected by both treatments, resulting in effects on species composition. Generally, species cover decreased with increasing glyphosate doses, although cover of *Festuca ovina* and *Euphorbia esula* forms exceptions. Increasing nitrogen, generally, resulted in increasing total plant cover and biomass, especially of fast-growing and competitive species as grasses and a few herbs such as *Tanacetum vulgare*.

The finding that increasing doses of glyphosate, especially in combination with the highest concentrations of nitrogen, resulted in an increased cover of bare soil and also in an increasing proportion of annuals is new. Boutin and Jobin (1998), however, found more what they called weedy and short-lived grassy-type plants in habitats adjacent to intensively farmed fields. An increasing cover of bare soil opens the vegetation and establishment of new species may occur. This also corresponds well with the finding of an increased proportion of annuals in plots treated by a combination of glyphosate and nitrogen as discussed below.

4.2 Effects of nitrogen and glyphosate on plant traits

Using trait-based approaches in studies linking plant diversity to ecosystem functioning or environmental stressors has been advocated by several studies (e.g., Dı´az and Cabido 2001, Lange et al. 2009). Dorrough and Scroggie (2008) demonstrated that plant traits, such as lifespan, growth form and origin, can be used to predict effects of stressors in pasture land. However, very few studies have been undertaken to establish the link between herbicide effects and plant traits, although multiple traits (e.g. growth habits, leaf morphology, lifespan, cuticular wax) have been suggested as key in explaining species sensitivity (Huangfu et al. 2009, Boutin et al. 2012). One recent study by Pellissier et al. (2014), also based on data from 'Kalø-plottet', found that an increase in nitrogen promoted an increase in the average specific leaf area (SLA) and canopy height, whereas glyphosate promoted a decrease in those traits. Additionally, the present analysis found an increase in Ellenberg N and Grimes C and a decrease in Grimes S with increasing nitrogen. With increasing doses of glyphosate, the present analysis also found an increase in Grimes S. The observed selection on plant traits may be caused by selection of a few species, e.g. glyphosate selects for *Festuca ovina* and nitrogen for *Elytrigia repens*. Although the height of the dominant plant species has been shown to respond to the treatment, plant height may not be considered a "robust" plant trait as it changes with environmental conditions for the individual species. Plant height is also used as effect measure (endpoint) in standard ecotoxicological testing. Decreasing SLA with increasing glyphosate doses corresponds with the finding of Boutin et al. (2012) that species with greater leaf surface area were more sensitive to glyphosate than those with less surface area. The increasing incidence of stress tolerance (Grimes S) and concomitant decrease in importance of the ruderal strategy (Grimes R) suggest that glyphosate acts on the plant community more as stress factor than as a disturbance, though an increased cover of bare soil was found especially for the combined nitrogen and

glyphosate treatment. The use of the trait-based approach seems to increase the general understanding of the processes behind the ecological responses to the treatments.

4.3 Effects of nitrogen and glyphosate on pollination

If floral traits involved in the attraction of pollinators are negatively affected, glyphosate and nitrogen exposure may lead to disruption of pollination interactions, resulting in reduced reproduction of obligatory insect pollinated plants. Furthermore, reductions or change in availability of floral resources due to herbicide exposure can have a negative impact on flower-visiting insect populations and, hence, have cascading effects on natural ecosystems. Results of the current study indicate that different plant traits are differently affected by exposure to glyphosate and nitrogen, as the nitrogen and glyphosate treatments have been shown to affect both species composition and plant flowering.

Regardless of the treatment, the plots have been found to be dominated by grasses (Strandberg et al. 2012). Generally, plant communities exposed to nitrogen are dominated by few, fast-growing, competitive species, mostly grasses (Kleijn and Snoeijing 1997, Kleijn and van der Voort 1997, Tsiouris and Marshall 1998, Vickery et al. 2001). These do not provide floral resources for flower-visiting insects (Proctor et al. 1996).

Whereas impact of sub-lethal dosages of herbicides on non-target plants, community composition and species diversity is well-documented (see above, section 4.1), less is known about impacts on the reproductive structures, including flowers, seeds and germination of seeds and subsequent impact for pollination. Reproductive structures not only have been found to be a more sensitive endpoint for risk assessment than vegetative endpoints in more than half of all available studies (EFSA PPR 2014), in addition, a few recent studies have documented that plants that have been exposed to herbicides flower less (Crone et al. 2009, Strandberg et al. 2013, Boutin et al. 2014) and flowering is delayed compared to un-exposed plants (Boutin et al. 2014).

For the two composite species *Tanacetum vulgare* and *Leucanthemum vulgare,* the two most heavily affected traits were floral density and flowering phenology, in turn leading to marked changes in plant-pollinator interactions. Glyphosate-treated plants were more stunted, but flower size did not change in *T. vulgare,* and only ray florets were affected in *L. vulgare*. Plots treated with nitrogen and increasing dosages of glyphosate generally had a low density of flowers, even at peak flowering. Reduction in density was most severe in the early-flowering *L. vulgare,* which started flowering shortly after glyphosate application, and flowering plants were completely lacking for many of the treated plots. However, even in *T. vulgare,* which were exposed two months prior to the natural flowering period, floral density of glyphosate treated plots was much reduced. In addition to reduction in density, flowering was significantly delayed in glyphosate treated plots of both species. In *L. vulgare,* the inflection point of the flowering curve was delayed 21.5 days per % application of glyphosate, while for *T. vulgare* the delay was 1.5 days per % application of glyphosate. Although this estimate of flowering delay for *L. vulgare* was based on a small sample size of flowers due to lack of flowering in treated plots, the estimate for *T. vulgare* is more robust. Results for the latter species indicate that spray drift dosages of 5 to 25% delay median flowering date by 7.5 and 40 days, respectively. Assuming that flowering delays and reductions in floral abundance is a general response of exposure to glyphosate, we may expect that plant-pollinator interactions of non-target plants are disturbed and flowering shifted towards late summer in conventionally farmed agricultural landscapes. Thus, we expect a general scarcity of floral resources for flower-visiting insets in early summer. For late-season plant species, such as *T. vulgare,* which has a natural flowering peak in late summer (inception point 5 August), peak flowering is shifted from a period of high insect activity to a less favourable period. In particular, high spray drift dosages may postpone flowering to autumn in late-flowering species, leading to a mismatch with the activity period of important pollinators.

Given that 61% of the area in Denmark is farmland and that 95% of this is conventional farming, it must be expected that a large proportion of natural and semi-natural areas embedded in agricultural landscapes are affected by herbicide spray drift. Advancement of flowering due to nitrogen application does not offset the effects of glyphosate.

4.4 Effects of nitrogen and glyphosate on soil fauna

The addition of N and glyphosate now for a period of 10 years expectedly could lead to cascading effects through the soil food web due to impacts of plant litter quality and biomass and the soil microbial community. Such effects would indirectly affect soil invertebrates and directly the key functional processes, in particular nutrient cycling. According to earlier toxicity studies, it is very unlikely that glyphosate in the applied concentrations would have direct effects on soil invertebrates (Eijsackers 1985, Dalby et al. 1995b, Pelosi et al. 2014), but as illustrated by the SEM model there could be indirect effects mediated through plant traits.

Earthworms

Based on earlier work investigating possible side effects of glyphosate on earthworms and other soil animals, it seems very unlikely that the negative correlation with earthworm biomass found in the SEM analysis is based on direct toxicity. Several studies show that glyphosate (or the compounds in the formulation) does not exert direct toxicity to earthworms in laboratory tests, let alone in field trials (Eijsackers 1985; Edwards 1992; Pelosi et al. 2014). It should also be noted that the mixed model ANOVA analysis did not indicate significant effects of glyphosate on any earthworm species, but there was significant interaction between nitrogen and glyphosate. This suggests that significant effects of glyphosate on earthworms are mediated through indirect effects. We noted in the SEM that deep root biomass had a significant negative effect on earthworm biomass. As such, this confirms our hypothesis that a high plant and root biomass might deplete bioavailable soil water and thereby impact upon endogeic earthworms. Indeed, we observed that the endogeic, A. tuberculata, was negatively influenced by nitrogen application (Table 3.5.2.1), and the SEM indicates that nitrogen application stimulated root growth which in turn would decrease the abundance of endogeic earthworms through reduction of soil water content. In the case of glyphosate the same indirect cascading effect was not seen. Glyphosate had a negative effect on deep root biomass, which, following the same argumentation as before, would in fact stimulate endogeic earthworms. However, we saw the opposite (i.e. the SEM indicated an overall negative effect of glyphosate on earthworms), which leaves our results on endogeic earthworms and glyphosate inconclusive. For one earthworm species, the surface dwelling and litter feeding D. octaedra, we observed a significant interaction between nitrogen application and glyphosate. In unfertilized plots glyphosate stimulated this species whereas glyphosate did not stimulate D. octaedra in nitrogen fertilized plots. One explanation of this could be that glyphosate application would increase the supply of high-quality litter, and thus improve the food conditions. In nitrogen amended plots the plant productivity and litter quality was perhaps already high, meaning that the effect on litter supply by glyphosate was less important and therefore not tracked in the abundance of litter feeding earthworms.

Nitrogen application caused a general shift towards earthworms feeding on litter. Thus, SEM relationship between nitrogen and the trait describing feeding preference of earthworms was in good agreement with the ANOVA analysis of single earthworm species. Here, we observed that litter feeding species, such as D. octaedra, were stimulated by nitrogen, whereas soil feeding species were reduced.

Collembola

SEM indicated that there was a negative relationship between glyphosate and habitat width of Collembola, whereas nitrogen had the opposite effect. Habitat width can be interpreted as a measure of adaptability to the habitat; high values indicate that a species is able to live in different

types of habitats, whereas low values indicate that a species is confined to narrow niches and few habitats. As outlined above, it is very unlikely that direct toxic effect of glyphosate is causing this effect (Eijsackers 1985). However, the SEM provides an interesting mechanistic explanation. Thus, there was a significant positive relationship between habitat width of Collembola and the trait defining dry matter content of plant leaves (LDMC). We may interpret this trait in terms of palatability to microarthropods: low LDMC plant litter is easily digested and has high nutritional value and vice versa. Coming back to glyphosate and nitrogen, SEM showed that glyphosate increased LDMC, whereas nitrogen had the opposite effect. Taken together, this strongly indicates that the negative and positive effects of glyphosate and nitrogen on habitat width, respectively, are mediated through effects on litter quality.

Deep root biomass was positively correlated with trophic position of Collembola. For this trait, we also saw indirect relationships with glyphosate and nitrogen, since these treatments had negative and positive effects on deep root biomass, respectively. Apparently, these latter effects on a plant trait cascade to the trait describing trophic position of Collembola. We may make the interpretation of this observation that both glyphosate and nitrogen addition shifts the Collembola community towards species that preferably feed on microbiota or on other soil animals through effects on deep root biomass. Some microarthropod species, such as Onychiuridae, do feed on fine roots (e.g. Ngosong et al. 2011). However, the SEM analysis indicated that increased root biomass shifts the Collembola community towards species that preferably feed on microorganisms or animals, i.e. the opposite of a stimulation of root feeders. Thus, we suggest that increased root biomass increases the rhizosphere community of microbes, protozoans and nematodes fuelling omnivorous microarthropods.

4.5 Effects of nitrogen and glyphosate on the soil foodweb

The soil food web structure was described by quantitative analyses of stable isotopes. This approach has been employed now for two to three decades in soil ecology and provides an overview of the trophic structure of the basic soil ecosystem elements, obviously as long as these elements have been sampled and included in the analysis (Spain et al. 1990, Briones et al. 1999b, Traugott et al. 2013). However, stable isotopes have not yet been employed in studies of ecosystem changes under the influence of externally imposed stressors, such as pesticides and fertilizers. The structure of the food web was in agreement with the commonly observed positions of the tropic groups in the soil ecosystem. Hence, the next step in the analysis was to detect changes in the foodweb structure brought about by the treatment of glyphosate and nitrogen.

Shifts in the trophic structure due to the treatments were evidenced from specific elements of the foodweb. The statistically significant shifts in the isotopic signature observed for plants and litter should expectedly cascade into the consumers, which it rarely did, but the dramatic change in C:N with N fertilization should be reflected in the consumers. We observed both stimulation and declines of microarthropod populations in response to the high N fertilization, thus, the coupling of soil fauna to the primary producers as a food base was of limited importance and species specific.

4.6 Effects of nitrogen and glyphosate on ecosystem function and properties

Nitrogen had a positive effect on plant biomass when the biomass of the roots was used as a proxy for the biomass of the total plant community. This effect of nitrogen was expected, since the soil of the experimental site was classified as nutrient poor sandy soil, which suggests that nitrogen most likely is one of the limiting resources for plant growth (Craine 2009). Oppositely, and in concordance with the hypothesis that herbicides have a disturbing effect on plant growth (Damgaard et al. Submitted-a), glyphosate had a negative effect on plant biomass and selected for plant species with an annual life cycle.

As expected, nitrogen selected for plant species with relatively thin leaves (high SLA, low LDMC). Thin leaves have a higher turnover rate and are more effective in competing for light, and consequently a higher Grime's C (Grime 2001, Craine 2009). Interestingly, glyphosate selected for plant species with thicker leaves with a higher dry matter content. Glyphosate is a systemic herbicide that is absorbed through the leaves and is translocated throughout the plant, and the increase in leaf thickness and LDMC could be a direct effect of glyphosates. On the other hand, the observed responses of the drivers on the mean leaf anatomy are at least partly explained by the preferential selection of *Festuca ovina* in plots with low nitrogen treatment and high glyphosate treatment, thus, the reported observations on the effect of glyphosate on leaf economy traits need to be confirmed in other long-term multi-species field experiments with glyphosate treatments (e.g. Pfleeger et al. 2012).

We observed that epigeic, litter feeding earthworms were stimulated by nitrogen, whereas endogeic species were suppressed. Endogeic and anecic earthworms are perhaps the most significant soil animals in temperate agricultural soils. These 'ecosystem engineers' can, by their tunnelling through the soil, increase soil porosity and average pore size (Edwards & Shipitalo, 1998). They ingest considerable amounts of soil and dead plant material, thereby contributing to the mixing of organic matter and mineral soil. This improves aggregate stability and increases the surface of organic material so that it is more readily colonized and decomposed by soil bacteria and fungi (Lavelle et al., 1997; Parmelee et al., 1998). The consequences of nitrogen application seem, therefore, to be a decreased influence of earthworms on soil physical conditions and with that of degradation of dead organic matter in the deeper soil horizons.

Collembola were generally stimulated by both glyphosate and nitrogen application, whereas we observed a negative effect of nitrogen on the Acari. Although these effects were statistically significant, the abundance of microarthropods did not change very much. A tentative conclusion might therefore be that ecological functions of microarthropods were not substantially altered due to glyphosate and nitrogen, however, shifts in community composition were clear.

5. Conclusion

The project has led to an increased understanding of the causal relationship between plant communities and the soil fauna at the ecosystem level and increased knowledge on how and by what mechanisms important drivers that are known to affect plant communities may affect pollination and the soil fauna. The combined use of plant trait and soil fauna trait data in a full-factorial field experiment of glyphosate and nitrogen has never been explored before. The focus on plant and soil fauna traits rather than species will enable a more robust description of the ecological processes at the functional level.

The empirical data of vegetation and soil fauna biodiversity and traits was linked to the underlying ecological processes at the functional level of the ecosystem using the modelling approach of structural equation modelling.

The benefit of the trait approach is its versatility and its ability to predict functional outcomes across habitats and locations. We are of the opinion that a proper valuation of ecosystem services must rely on ecosystem descriptions of more general applicability to be integrated into economic models, and in this respect we use the ecosystem service in a rather restricted sense by focussing on the ecological functions and structures that may be of practical importance of e.g. farmers. Hence, our contribution to implementation of ecosystem services into Danish environmental management practices is to develop the scientific basis for incorporating the natural capital into resource- and land-use decisions on a large scale.

6. Perspectives

6.1 Perspectives - scientific

The aim of the study was to improve our understanding of and be able to quantify and predict the effects of glyphosate and nitrogen and their interaction on small terrestrial biotopes in the agricultural landscape, e.g. hedgerows and field margins. For both vegetation and soil fauna, the effects were assessed at the ecosystem level by measuring biodiversity and functional traits.

We and others have demonstrated strong selection forces of glyphosate and nitrogen on plant species composition (Holst et al., 2008; Damgaard et al., 2011; Strandberg et al., 2012; Damgaard et al., 2013; Damgaard et al., in press), as well as on plant traits (Pellissier et al., 2014). However, since glyphosate is known to modify the interspecific interactions (Damgaard et al., 2011; Damgaard et al., in press), the effect of plant traits on the interspecific interactions at the different treatments has to be studied in order to make a credible predictive population ecological model of the selective effects of herbicide and nitrogen on plant traits. Furthermore, in order to make a general predictive model the investigated herbicide effects have to be generalized to herbicides with other modes of action. Such a generalization will also allow an increased understanding of the underlying mechanistic causes of the trait based selection.

The empirical data of vegetation and soil fauna biodiversity and traits was linked to the underlying ecological processes at the functional level of the ecosystem using the modelling approach of structural equation modelling. This will potentially enable us to develop quantitative tools for predicting the effects of glyphosate and nitrogen and their interaction on vegetation and soil fauna traits at the ecosystem level and the connected ecosystem properties and functions in small terrestrial biotopes in the agricultural landscape.

6.2 Perspectives – administrative

The trait-based approach clearly has some advantages with respect to understanding pesticide effects on plant communities although a couple of disadvantages also have been identified (EFSA 2014). The advantages include 1) a simplified description of communities, 2) utilization of simple and relatively easily accessible attributes, 3) better linkage of plant diversity to processes and functions of targeted ecosystems, 4) reduced constraints related to scale and geographic differences, and 5) facilitation of comparisons of communities with different species pools (EFSA 2014). The recognized disadvantages include phenotypic plasticity of some plant traits and intercorrelation among traits. Irrespective the immediate number of advantages, the conclusion of the present study that nitrogen promoted an increase in traits, i.e. the average specific leaf area (SLA) and canopy height, that glyphosate promoted a decrease in, may question the applicability of the trait-based approach for evaluation of habitat quality within agricultural areas as those habitats normally are highly affected by nitrogen as well as pesticides. The question is how general these opposite effects of nitrogen and herbicide are. Among the disadvantages of the approach the phenotypic plasticity may also pose a problem as plant height has been recognized as a relatively plastic response.

Specific protection goals have been defined for non-target terrestrial plants for off- and in-field habitats (EFSA 2014). Among others, the specific protection goals should take into account pollination, food webs, nutrient cycling and biodiversity. The finding of the present study that floral density and flowering period of two melittophilous plant species, Tanacetum vulgaris and

Leucanthemum vulgare, was highly affected by low doses of glyphosate independently of nitrogen level might have important implication for both biodiversity and pollination.

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Appendix 1: Plant Traits

The selected plant traits used in the analysis are listed in Table A1. The investigated species specific plant traits were either measured characters (e.g. specific leaf area), life history characters (e.g. age at the first flowering event), or index values assessed by experts. The quantitative traits include leaf-height-seed (LHS) traits (Westoby 1998), Ellenberg plant species indicator values (Ellenberg 1979), Grime's plant species CSR strategies (Grime 2001) and life history traits. We find that it is meaningful to base the investigation on these groups, although some of them are based on expert opinions rather than measured traits. The leaf, height and seed mass traits implied by the LHS strategy are basic plant traits that are simple to measure. This contrasts to the Ellenberg ecological indicator values (EIV), which are semi-quantitative composite traits that cannot be strictly understood as basic plant traits. Rather, they represent an assembly of structural and physiological traits that together augment tolerance to soil acidity, salinity, drought tolerance etc. EIVs were the first ecological indicator model applying values to the flora of Germany and have a long tradition in interpretation and understanding of plant communities. The interpretation of the Ellenberg indicator values is not always simple (Ertsen et al. 1998, Schaffers and Sýkora 2000); for example, the moisture indicator Ellenberg F indicates a combination of high groundwater table and soil ability to retain water, and correlation is strongest to the soil moisture content in a dry period (Schaffers and Sýkora 2000). Likewise, the CRS-strategies suggested by Grime (2001) are also composite plant traits. The CSR-triangle theory predicts that the degree of competition, stress and disturbance characterizing a habitat will determine which species will occupy the habitat. In this scheme, the plant species have values in the three dimensions Competition, Stress and Ruderal (disturbance). Generally, Grime's C and Ellenberg's N indicators are positively correlated, and an increase in both indicators suggests an increase in plant biomass and general competitive ability (Timmermann et al., unpublished). Like the EIVs, the CSR-triangle theory has been subject to criticism, e.g. for the lack of obvious tests to apply to validate the values (Wilson and Lee 2000). CSR- and EIV-values have been treated as traits in comparable analyses by some authors (e.g. Pywell et al. 2003), who for simplicity refer to these measures as traits. We have chosen to follow the example of Pywell (2003) in this study.

Plant trait	Unit / Interval	Data source
Ellenberg L	1-9	LEDA
Ellenberg T	1-9	LEDA
Ellenberg K	1-9	LEDA
Ellenberg F	1-12	LEDA
Ellenberg R	1-9	LEDA
Ellenberg N	1-9	LEDA
Grime C	0-12	LEDA
Grime S	0-12	LEDA
Grime R	0-12	LEDA
Annual	Binary	LEDA
Seed mass	g	LEDA
SLA	m^2/g	LEDA
Canopy height	m	LEDA
LDMC	g/m^3	LEDA
Leaf mass	g	LEDA
Leaf size	m^2	LEDA
Dicotyledons	Binary	LEDA
Specific root length, SRL	m/g	ECOMARG
Fine root diameter	Mm	ECOMARG
Root depth distribution	g/m^3	ECOMARG
95% rooting depth	m	ECOMARG

TABLE A1.1
LIST OF TRAITS THAT WILL BE USED WITHIN ECOMARG. THE SPECIES SPECIFIC PLANT TRAIT VALUES WERE FOUND EITHER IN THE LEDA DATABASE (KLEYER ET AL. 2008) OR MEASURED IN THE PROJECT.

Appendix 2: Soil Fauna Traits

The collembolan trait database including the species encountered at the ECOMARG study site was a subset of a larger database available from SoilBioStore.AU.dk. A set of 17 traits was created for 24 collembolan species, only omitting *Isotomodes bisetosus* and *Stenaphorura quadrispina*, as they were anyway rare. Moreover, unidentified species of the groups Entomobryidae and Symphypleona, as unequivocal traits, need a species name to be properly defined. The trait values were scaled to a range of 0-1 to avoid an unintended weighing due to the categorical or continuous levels that were initially chosen for the traits.

Main category	Label	Categorial and continuous levels	Acronym	Incl.
Taxonom	*Species taxonomical name*	Order, Family, Genus, Species, Acronym, Source		
Morphological trait	*No. of ocelli*	0 - 8	ocel	+
	Body size (max.)	mm, to the nearest 0.1 mm	length	+
	Body pigmentation level (max)	0 white, 1 lightly, 2 intensely	pigment	+
	Body pigmentation pattern	0 absent, 1 present, 2 spotted	pattern	+
	Modified hairs or scales	0 absent, 1 present	setae	+
	Furca development	0 absent, 1 reduced, 2 fully developed short, 3 f.d. long	furca	+
	Antenna estimated length	0 short, 1 medium, 2 long	ant_len	+
	Antenna: Head Ratio (max)		ant_head	+
	Antenna/ body ratio		ant_body	
Ecological trait	*Life form (morhological) sensu Gisin (1943, 1947, 1948)*	1 epedaphic/ Atmobios; 2 hemiedaphic-xerophile 3 hemiedaphic-mesophile; 4 hemiedaphic hydrophile; 5 euedaphic	LF_gis	+
	Life form (Rusek 1989)	1 Atmobios 2 Ba epigeonts 3 Bb hemiedaphobionts 4 Bc1a large euedaphobionts with furca 5 Bc1b large euedaphobionts without or reduced furca 6 Bc2a medium euedaphobionts with furca 7 Bc2b medium euedaphobionts without or reduced 8 Bc3a small euedaphobionts with furca 9 Bc3b small euedaphobionts without or reduced furca	LF_rus	+
	Vertical habitat preference lifeform	1 epedaphic, 2 hemiedaphic, 3 euedaphic	LF_gis_3	+
	Habitat preference	1 plant, 2 soil surface, 3 soil 0-10 cm depth		
	Moisture preference	0 xeroresistant, 1 xero-mesophilic, 2 indifferent, 3	moistprf	+
	Habitat width	0 steno, 1 steno/eury, 2 eury and eury/syn	eurytrf	+
	Trophic position	1 herbivore/ pollen feeder, 2 microbivore, 3 predator and microbivore	trp_pos	+
	Mode of reproduction	0 generally parthenogenetic, 1 bisexual, 2 both modes occur	rpr_mode	+
	Phenology	1 univoltine, 2 bivoltine, 3 multivoltine	phenol	+
	Mouthparts	1 sucking, 2 grinding, 3 piercing	teeth	+
	*Metabolic rate corrected for weight**	1 low, 2 medium, 3 high	metablrt	
	Weight of progeny relative to weight	Quantitative ratio	prog_rt	

TABLE A2.1
THE MORPHOLOGICAL AND ECOLOGICAL COLLEMBOLAN TRAITS INCLUDED IN THE TRAIT DATABASE. +: INCLUDED IN TRAITS SCORE MATRIX.

The abundance data of individual species were translated into a matrix of weighted trait scores, T_{ji}, of trait j of n species in m samples:

$$T = \sum_{i}^{n} \quad \sum_{j}^{m} p_i t_j$$

where p_i is the proportion of the population abundance of a species in a sample i.

TABLE A.2.2.
EARTHWORM TRAIT VALUES

	MORFOLOGICAL					ECOLOGICAL												
Traits	Length	Post - clitellar diameter	Pre-clitellar diameter	Pigmen-tation	Prostomium	Ecological category	Burrow type	Burrow max. depth	Burrow direction	Trophic position	Mode of reproduction	Fecundity	Cocoon cold tolerance	Worm cold tolerance	Age at maturity	pH preference	Drought resistance	Resistance to flooding	Food type
Units/ levels	Max length at maturity (cm)	Max diameter at maturity (mm)	Max diameter at maturity (mm)	A: Absent; P: Present	E: Epilobous; T: Tanylobous	A: anecic; E: endogeic; P: epigeic; L: limnic	P: permanent; T: transient	cm	0: No burrow; 1: Horisontal; 2 Vertical	δ index 0: Litter feeding 1: Soil feeder	P: Parthenogenetic; S: Sexual; F: facultative parthenogenetic	Eggs/cocoons at optimum temperature	Intolerant;Tolerant Highly tolerant	I: intolerant; T: tolerant; H: highly tolerant	Days at 15°C		I: intolerant; T: tolerant; H: highly tolerant	I: intolerant; T: tolerant; H: highly tolerant	L: litter; S: SOM; M: mixed SOM and litter
Aporrectodea longa	17	9	9	P	E	A/E	P	100	1.5	1	S	15	T	I	120	6.5	H	H	M
Aporrectodea tuberculata	15	8	8	A	E	E	T	70	1	0.8	S	60	T	T	120	6.5	H	T	S
Dendrobaena octaedra	6	4	5	P	E	P	T	10	0	0	P	80	H	H	70	5	H	T	L
Dendrodrilus rubidus	10	5	5	A	E	P	T	10	0	1	F	100	H	T	60	5	T	T	L
Lumbricus rubellus	13	5	5	P	T	P/E	T	10	0.5	0.6	S	50	T	I	70	6.5	T	T	L
Lumbricus terrestris	30	10	10	P	T	A	P	200	2	0.1	S	40	I	I	150	6.5	T	T	L

Appendix 3: Fit of the structural equation mode

```
lavaan (0.5-16) converged normally after 204 iterations

  Number of observations                           120

  Number of missing patterns                         4

  Estimator                                         ML
  Minimum Function Test Statistic              256.274
  Degrees of freedom                                28
  P-value (Chi-square)                           0.000

Model test baseline model:

  Minimum Function Test Statistic              869.471
  Degrees of freedom                                90
  P-value                                        0.000

User model versus baseline model:

  Comparative Fit Index (CFI)                    0.707
  Tucker-Lewis Index (TLI)                       0.059

Loglikelihood and Information Criteria:

  Loglikelihood user model (H0)              -1823.488
  Loglikelihood unrestricted model (H1)      -1695.351

  Number of free parameters                         86
  Akaike (AIC)                                3818.976
  Bayesian (BIC)                              4058.700
  Sample-size adjusted Bayesian (BIC)         3786.809

Root Mean Square Error of Approximation:

  RMSEA                                          0.261
  90 Percent Confidence Interval          0.232  0.290
  P-value RMSEA <= 0.05                          0.000

Standardized Root Mean Square Residual:

  SRMR                                           0.091

Parameter estimates:

  Information                                 Observed
  Standard Errors                             Standard

                   Estimate  Std.err  Z-value  P(>|z|)   Std.lv  Std.all
Regressions:
  Annuals ~
```

```
  Nit               0.043    0.253    0.170    0.865    0.043    0.015
  Gly               0.360    0.106    3.400    0.001    0.360    0.296
Dichot ~
  Nit               0.137    0.160    0.855    0.393    0.137    0.078
  Gly              -0.033    0.067   -0.486    0.627   -0.033   -0.044
SLA ~
  Nit               0.388    0.028   13.993    0.000    0.388    0.676
  Gly              -0.124    0.012  -10.630    0.000   -0.124   -0.513
LDMC ~
  Nit              -0.902    0.215   -4.197    0.000   -0.902   -0.316
  Gly               0.565    0.090    6.264    0.000    0.565    0.471
Grime_C ~
  SLA               6.479    0.331   19.586    0.000    6.479    1.005
  LDMC              0.175    0.092    1.906    0.057    0.175    0.135
  Annuals           0.117    0.039    2.994    0.003    0.117    0.092
  Dichot            0.415    0.116    3.564    0.000    0.415    0.197
Root_top ~
  Nit               0.136    0.070    1.943    0.052    0.136    0.217
  Gly              -0.063    0.032   -1.981    0.048   -0.063   -0.240
  Annuals           0.016    0.021    0.731    0.465    0.016    0.072
  Dichot           -0.132    0.045   -2.947    0.003   -0.132   -0.370
Root_bottom ~
  Nit               0.262    0.101    2.582    0.010    0.262    0.284
  Gly              -0.125    0.046   -2.709    0.007   -0.125   -0.322
  Annuals           0.059    0.031    1.892    0.059    0.059    0.186
  Dichot           -0.069    0.064   -1.084    0.279   -0.069   -0.131
Worms_biomass ~
  Nit               0.107    0.128    0.836    0.403    0.107    0.090
  Gly              -0.118    0.057   -2.089    0.037   -0.118   -0.237
  Root_top         -0.076    0.242   -0.313    0.755   -0.076   -0.040
  Root_bottom      -0.376    0.162   -2.317    0.021   -0.376   -0.292
  LDMC             -0.058    0.046   -1.244    0.213   -0.058   -0.138
Worms_feeding ~
  Nit               0.120    0.053    2.266    0.023    0.120    0.250
  Gly              -0.003    0.023   -0.109    0.913   -0.003   -0.013
  Root_top          0.027    0.097    0.278    0.781    0.027    0.035
  Root_bottom      -0.026    0.066   -0.389    0.697   -0.026   -0.049
  LDMC              0.009    0.019    0.450    0.653    0.009    0.051
Vertical_habitat ~
  Nit               0.135    0.104    1.295    0.195    0.135    0.143
  Gly               0.073    0.045    1.609    0.108    0.073    0.184
  Root_top         -0.048    0.203   -0.235    0.814   -0.048   -0.032
  Root_bottom      -0.120    0.140   -0.853    0.393   -0.120   -0.117
  LDMC              0.008    0.037    0.206    0.837    0.008    0.023
Habitat_width ~
  Nit               0.246    0.110    2.241    0.025    0.246    0.238
  Gly              -0.137    0.053   -2.581    0.010   -0.137   -0.315
  Root_top         -0.429    0.219   -1.959    0.050   -0.429   -0.260
  Root_bottom       0.202    0.157    1.284    0.199    0.202    0.180
  LDMC              0.109    0.042    2.624    0.009    0.109    0.301
  Annuals           0.043    0.032    1.340    0.180    0.043    0.120
Trophic_position ~
  Nit               0.011    0.017    0.655    0.512    0.011    0.072
```

```
    Gly               0.004    0.007    0.559    0.576    0.004    0.064
    Root_top         -0.062    0.036   -1.706    0.088   -0.062   -0.252
    Root_bottom       0.074    0.024    3.147    0.002    0.074    0.443
    LDMC              0.002    0.006    0.358    0.721    0.002    0.040

Covariances:
  SLA ~~
    LDMC             -0.234    0.052   -4.513    0.000   -0.234   -0.452
  Vertical_habitat ~~
    Habitat_width    -0.143    0.060   -2.375    0.018   -0.143   -0.236
  Grime_C ~~
    Worms_biomass     0.062    0.093    0.664    0.507    0.062    0.067
    Worms_feeding    -0.010    0.039   -0.250    0.802   -0.010   -0.025
    Vertical_hbtt    -0.050    0.076   -0.659    0.510   -0.050   -0.066
    Habitat_width     0.106    0.083    1.282    0.200    0.106    0.137
    Trophic_postn     0.004    0.012    0.335    0.738    0.004    0.036
  Worms_biomass ~~
    Worms_feeding     0.082    0.036    2.288    0.022    0.082    0.220
    Vertical_hbtt     0.040    0.068    0.595    0.552    0.040    0.056
    Habitat_width     0.088    0.072    1.221    0.222    0.088    0.120
    Trophic_postn    -0.012    0.012   -0.972    0.331   -0.012   -0.108
  Worms_feeding ~~
    Vertical_hbtt     0.029    0.028    1.037    0.300    0.029    0.096
    Habitat_width     0.036    0.030    1.202    0.229    0.036    0.115
    Trophic_postn     0.000    0.005    0.018    0.986    0.000    0.002
  Vertical_habitat ~~
    Trophic_postn     0.016    0.009    1.756    0.079    0.016    0.181
  Habitat_width ~~
    Trophic_postn    -0.022    0.009   -2.369    0.018   -0.022   -0.248

Intercepts:
    Annuals          99.893    0.343  291.428    0.000   99.893   40.541
    Dichot            2.102    0.217    9.702    0.000    2.102    1.408
    SLA               2.005    0.038   53.274    0.000    2.005    4.105
    LDMC             27.849    0.292   95.516    0.000   27.849   11.461
    Grime_C         -27.159    5.199   -5.224    0.000  -27.159   -8.627
    Root_top          2.771    2.092    1.324    0.185    2.771    5.203
    Root_bottom      -4.095    3.082   -1.328    0.184   -4.095   -5.226
    Worms_biomass     4.221    1.358    3.109    0.002    4.221    4.181
    Worms_feeding     0.280    0.558    0.501    0.616    0.280    0.684
    Vertical_hbtt     8.381    1.098    7.632    0.000    8.381   10.437
    Habitat_width     3.357    3.738    0.898    0.369    3.357    3.818
    Trophic_postn     6.654    0.183   36.370    0.000    6.654   50.775

Variances:
    Annuals           5.537    0.715                      5.537    0.912
    Dichot            2.212    0.286                      2.212    0.992
    SLA               0.067    0.009                      0.067    0.280
    LDMC              4.006    0.517                      4.006    0.679
    Grime_C           0.969    0.127                      0.969    0.098
    Root_top          0.222    0.042                      0.222    0.783
    Root_bottom       0.495    0.099                      0.495    0.807
    Worms_biomass     0.878    0.121                      0.878    0.862
```

Worms_feeding	0.158	0.020	0.158	0.944
Vertical_hbtt	0.595	0.078	0.595	0.923
Habitat_width	0.618	0.089	0.618	0.799
Trophic_postn	0.013	0.002	0.013	0.768

TABLE A3.1
STRUCTURAL EQUATION MODEL OF COMMUNITY WEIGHTED AVERAGE TRAIT VALUE AND OTHER ECOLOGICAL VARIABLES. THE NITROGEN AND GLYPHOSATE TREATMENTS ARE EXOGENOUS VARIABLES. OUTPUT STATISTICS FROM "LAVAAN" (ROSSEEL 2012).

The effect of glyphosate and nitrogen on plant communities and the soil fauna in terrestrial biotopes at field margins

The aim of the study was to improve our understanding of the effects of glyphosate and nitrogen and their interaction on small terrestrial biotopes in the agricultural landscape,

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