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The combined effect of nitrogen and glyphosate on the competitive growth, survival and establishment of *Festuca ovina* and *Agrostis capillaris*

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ABSTRACT

In order to study the combined effect of nitrogen and glyphosate on biodiversity in agricultural areas, a replicated long-time field experiment with glyphosate and nitrogen treated plots was set-up. The experiment allowed a quantitative estimation of the effect of glyphosate and nitrogen on competitive growth, survival and establishment of the dominating species during and between growing seasons. It was found that the observed ecological success of *Festuca ovina* relative to *Agrostis capillaris* in glyphosate treated plots was primarily due to altered competitive plant growth during the growing season rather than an immediate die back following spraying. Overall, interaction of herbicide and fertilizer on plant competitive growth, survival and establishment were demonstrated, and it was suggested that positive interactions between glyphosate and nitrogen may be important for the ecological success of *A. capillaris* in field margins. The used method of combining pin-point data and Bayesian state-space competition models may be applied in other ecological studies. For example, the method may be used in predicting the effects of altered weed control strategies on the botanical composition of the agro-ecosystem.

1. Introduction

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, 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. In the agro-ecosystem relatively small natural and semi-natural biotopes in the neighbourhood of agricultural fields play a relative large role for maintaining biodiversity by providing key habitats for both fauna and flora, corridors for spreading, and an important source of ecosystem services such as pollination. In order to manage biodiversity in agro-ecosystems it will consequently be valuable to be able to predict the effect of e.g. a non-spayed border zone in field margins on biodiversity in the neighbouring natural and semi-natural biotopes.

Nitrogen addition experiments have shown variable effects on the botanical composition of natural and semi-natural habitats within the agricultural areas; from no apparent effects of adding nitrogen on hedge bank vegetation (Boatman, 1994; Theaker et al., 1995) to reduced species richness and changes in ecosystem composition and functioning in grassland communities (e.g. Mountford et al., 1993; Bobbink et al., 1998; Gough et al., 2000; Clark and Tilman, 2008). The observed differences may be caused by differences in the pre-addition nitrogen level of the soil as well as a variable response among different types of plant communities.

Different studies have pointed at herbicide spray drift as a major factor affecting both flora and fauna of field boundaries and hedgerows (e.g. Bhatti et al., 1995; Aude et al., 2003; Holst et al., 2008). Jensen (2006) and Jensen et al. (2007) demonstrated that evaporated herbicides have the potential to affect plants significantly, and that low dosages of herbicides may both reduce the number of species and affect the species composition, and Marrs et al. (1989, 1991) found that spray drift resulted in sublethal, but significant effects, such as plant damage and flower suppression on single plant species and argue that spray drift may have long-term impacts on plant community structure. This hypothesis was corroborated in a competition experiment between *Capsella bursa–pastoris* and *Geranium dissectum* at variable doses of the herbicide mecoprop-P, where it was predicted that the two species were more likely to coexist at low doses of the herbicide (Damgaard et al., 2008).

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As briefly summarized above, there is substantive and 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. We were only able to find three published studies on the effects of combined additions of glyphosate and nitrogen: one on exposure of woodland plants (Gove et al., 2007) and two on exposure of experimentally established vegetation mimicking effects on field margins (Perry et al., 1996; Bruus Pedersen et al., 2004). All the studies show significant effects of glyphosate concentrations equivalent to those measured at doses of 1–25% of full application rate, and the responses of the vegetation was affected by application of fertilizer. Gove et al. (2007) found increased mortality, reduced biomass and reduced fecundity of glyphosate and nitrogen in all six species tested, both in greenhouse experiments, where plants were grown separately and exposed when they were six weeks, and when transplanted into plots in woodland margins. Perry et al. (1996), who only reported first year results, found that although the individual species (three monocots and three dicots) responded differently to the treatments, both fertilizer and glyphosate affected the community significantly. Concurrently, Bruus Pedersen et al. (2004) found species dependent responses to glyphosate and interactions between nitrogen and glyphosate on species richness and total biomass. In addition, efficacy studies of herbicides indicate that the herbicide susceptibility of the different weed species was influenced by the nitrogen level (Cathcart et al., 2004). Recently, the efficacy of the herbicide tribenuron-methyl on *Tripleurospermum inodorum*, *Anagallis arvensis* and *Chenopodium album* at different soil nitrogen levels was compared and a reduced effect on the two former species at very low nitrogen levels was observed (Sønderskov, unpublished data), while no interactions were observed for the latter. Similarly, soil nitrogen levels only had minor effects on growth delay and phenological development following application of sub-lethal doses of tribenuron-methyl (Sønderskov, unpublished data). As a response to this important knowledge gap, a replicated long-time field experiment with glyphosate and nitrogen treated plots was set-up (Bruus Pedersen et al., 2004; Holst et al., 2008), and this study reports on the findings.

Often the composition of plant communities has been found to depend on inter-specific competitive relationships (e.g., Weiher et al., 1998; Silvertown et al., 1999; Gotelli and McCabe, 2002), and it may be expected that the effects of sub-lethal levels of herbicides and fertilizer on plant community composition are mediated by altered inter-specific competitive interactions. This hypothesis is corroborated by the finding that the availability of nitrogen affected the competitive interactions between two *Eriophorum* species (McGraw and Chapin, 1989), as well as by a green house competition experiment, where it was found that the competitive interactions between sheep's fescue (*Festuca ovina*) and common bent grass (*Agrostis capillaris*) depended on the level of the herbicide glyphosate, in such a way that the competitive effect of *F. ovina* increased with the level of glyphosate (Holst et al., 2008). Furthermore, it was observed that *F. ovina* was relatively more abundant than *A. capillaris* in glyphosate treated plots (Holst et al., 2008), and these observations led us to investigate the effect of glyphosate and nitrogen application on the competitive growth, survival and establishment of *F. ovina* and *A. capillaris* under field conditions. Consequently, the aim of the present study is to model the competitive growth, survival and establishment of *F. ovina* and *A. capillaris* under field conditions, and to test whether the competitive growth, survival and establishment of the two plant species are affected by the levels of glyphosate or nitrogen, and whether there is an interaction effect between nitrogen and glyphosate on the competitive growth, survival and establishment.

2. Materials and methods

2.1. Field experiment

The field experiment was designed to study the ecological processes, including establishment, survival and competitive interactions, in a semi-natural ecosystems affected by herbicide (glyphosate) and fertilizer (nitrogen) in a relatively realistic way. Abundance and species composition, therefore, were not controlled, except for the removal of woody species (trees and bushes) every year prior to herbicide application.

The selected area was a former agricultural field on dry, nutrient poor sandy soil. The field laid fallow for a couple of years prior to the start of the experiment in 2001. The field is quadrangular and surrounded by forest on two sides (south and west) and separated from the neighbouring fields by 5 m broad hedgerows on the other sides.

In 2001, the area was deep ploughed down to 60 cm to minimise establishment from the soil seed bank and prepared for the experiment by harrowing and rolling. Thirty-one selected grassland plant species covering different life form strategies (CRS strategies sensu Grime, 2001) were sown in spring 2001 (Bruus Pedersen et al., 2004).

The experimental manipulations were set up as a complete randomized block design with 10 replicates of twelve treatments. Each replicate plot was 7 m × 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.

2.2. Treatments

The treatments included 4 glyphosate treatments (0; 14.4; 72 and 360 g a.i./ha RoundupBio®, Monsanto Crop Science, Denmark A/S, 360 g/L glyphosate as a isopropylamine salt). The applied doses were equal to 0, 1, 5 and 25%, respectively, of the dose recommended for several purposes, including both pre- and post-harvest treatments (Cordsen Nielsen et al., 2008), and 3 nitrogen treatments (0, 25 and 100 kg N/ha). All 120 plots received phosphorus (53 kg/ha), potassium (141 kg/ha), sulphur (50 kg/ha) and copper (0.7 kg/ha) every year.

For the herbicide applications, spraying equipment for experimental applications was used. The boom was 3 m with 0.5 m between the nozzles that were Lurmark Low-drift LD 015 Green nozzles operated at a pressure of 2.0 bar. The wind speed on the days selected for spraying was very low (0–2 m/s). There was no rain during the days following the application. The amount of fertilizer was weighed individually for each plot and spread by hand. The plots were treated by glyphosate for the first time 24 August 2001, when the vegetation had become established at the plots. Since then, glyphosate and fertilizer treatments were carried out once every year in spring (Strandberg et al., in press).

2.3. Sampling

In order to study the species interactions and the influence of nitrogen and glyphosate on the interactions of the two perennial grass species *F. ovina* and *A. capillaris*, which are known to differ in their responses to both treatments (Holst et al., 2008), one permanent 0.5 m × 0.5 m quadrate was placed within each of the 120 plots in June 2007. The quadrate was not placed randomly, but in such a way that both *F. ovina* and *A. capillaris* were noticeably abundant in the quadrate. Plant cover and vertical density of all vascular plant species within the quadrates were measured non-destructively by the pin-point method (Levy and Madden, 1933; Kent and Coker, 1992), using a pin-point frame with the same dimension as the