Summary

Introduction
Biodiversity in the sense of species richness may play a crucial role for maintaining ecosystem processes and therefore also for maintaining biodiversity itself. Pollination by animals is such a process. Worldwide species within nearly all taxonomical groups have declined or disappeared. This may be harmful for the functioning of ecosystems, thereby leading to further declines of biodiversity.

Biotic pollination involves two parties or communities, plants and animals, that mutually benefit from each other. Animals actively or passively transfer pollen grains needed for the pollination and seed set of plants. Plants provide animals with food, shelter or pheromones in the flowers. Pollination is important for the majority of wild plants and many crops: without pollination plant species and harvests will decline. This thesis is about the importance of biodiversity for the pollination of insect-pollinated plants.

Pollination systems can be classified into types of plant species with similar flowers that are visited by similar pollinator taxa. These types are called pollination syndromes. Plants that are pollinated by one or few pollinator species, and animals that confine their menu choice to one or few plant species, are called specialists. Plants pollinated by many pollinator species, and insects foraging on many plant species, are generalists. The majority of plant-pollinator interactions are unspecialised. The distribution of interactions between plants and flower visitors is asymmetrical: specialist plant species are visited by mainly generalist animals, and specialist animals visit mainly generalist plants. One-to-one relationships between single plant and animal species are extremely rare, particularly in temperate climates. In the study area of this thesis (the Netherlands, northwest Europe), only insects occur as pollinators. The majority belong to Diptera (flies, including syrphids), Lepidoptera (butterflies and moths) and Hymenoptera (mainly bees and bumblebees).

The role of plant and insect diversity for pollination is largely unknown. Plant diversity can have positive (facilitation) and negative (competition) effects on pollination of a plant species: e.g. several plant species together can attract more pollinators than one, but plants can also compete for pollinators. The higher the insect diversity, the higher will be the chance that a certain plant species is visited by its appropriate pollinator(s). Because most pollination interactions are unspecialised, the relation between diversity and pollination will not be straightforward: the extinction of a single species at one of the interacting levels does not directly lead to one extinction at the other level.

In Europe, including the Netherlands, most ecosystems are semi-natural and a result of centuries of extensive farming practices. During the second half of the 20th century, land use changed drastically and agriculture intensified. Many habitats declined, were deteriorated or became fragmented, resulting in the decline of plant and animal species. Two
important flower-visiting insect taxa, butterflies and bees, are among the most negatively affected groups. In intensified agricultural landscapes, habitat remnants are important for preserving biodiversity outside natural reserves, and may be stepping stones between reserves. In the Netherlands, road verges, ditches and field margins are the main habitat remnants.

Questions and methods
The main question of this thesis was:
Is a high biodiversity important for the pollination of entomophilous wild plant species in agricultural, fragmented landscapes?

Biodiversity was studied in various ways, from landscape and community level to pollination and seed set at the level of individual species or patches. The complete sequence from insect visitation and behaviour, via actual pollination until seed set and germination was included. How finally seed set affects plant species numbers, and thus community composition, was out of the scope of this study.

The research area was in the north of the province of Drenthe in the Netherlands. Most of the research activities were performed in road verges and ditch banks, situated in matrices with various types of land use. Land use varied between heavily fertilised and sprayed intensive grassland and arable land, intensively or extensively grazed meadows, and semi-natural plant species-rich habitats (mostly in natural reserves of the stream valley of the Drentse Aa).

Both field surveys (descriptive analyses) and experiments (garden and field) were used. In the field surveys, the diversity of flowering plants and their flower visitors were measured with transects of road verges and ditch banks of 100 m. Insects on all flowers were counted every two weeks at 51 sites from May-October 2000 and at 18 sites from May-August 2001. The total number of flowering plant species found was 97 from 24 families in 2000 and 74 species from 20 families in 2001. The number of insect species was 361 from 9 orders in 2000, and 204 species from 8 orders in 2001. The large data set was used in the chapters 2 - 5 and 8. Experiments with potted plants of a number of selected species in 2000 - 2003 were used in the chapters 5, 6 and 7. With potted plants the number and arrangement of flowers can be controlled, and the plants can be placed into various environments. Furthermore, the growing circumstances are equal for the plants. Visitation rate (the number of visits a flower receives per unit time) and pollination, and seed set were measured in the experiments.

Landscape, plant and insect diversity, and flower visitation
In the first three chapters, processes were studied at community level. In chapter 2, the functional diversity of the plant and insect species was described: what types of pollination systems or syndromes can be found, and what are the degrees of specialisation of the plant species? The use and application of pollination syndromes is frequently criticised in literature: syndromes are often applied without field data, and syndromes are said to indicate that pollination interactions of plants and animals are specialised, while the majority
are generalised. The goals were (1) to determine classes of plant species with similar frequencies of flower visiting insect taxa using cluster analysis, and (2) to investigate how existing pollination classifications are reflected in those classes. The majority of the plant species appeared to be generalists and not more than a third can be regarded as ecologically specialised. Fourteen classes could be identified. The proportion of flies, syrphids and solitary bees on the one hand, and the proportion of bumblebees, *Rhingia campestris* (a long-tongued syrphid) and honeybees on the other were the main characteristic insects for the two major two groups of classes. The accessibility of nectar and pollen was the most important feature that determined the differences of proportions of the visitor groups between the classes. Two of the known pollination syndromes were applicable for less than half of the plant species: the syndrome of flies (two classes with generalist plants) and the syndrome of bees (one class of specialist plants).

In chapter 3 species richness and abundance of plants and insects were quantified in relation to the type and intensity of land use ("landscape diversity") and each other. The diversity of plants and solitary bees at a site was affected by the surrounding type of land use, while total insect diversity was not. Plant and solitary bee diversity were lowest at sites with the highest agricultural intensity. Insect diversity was positively related with plant diversity: the more plant species and flowers at a site, the more insect species and individuals could be found. Fragmentation and land use affect plant species richness and abundance, and together with site management these affect insect species richness and abundance. Particularly solitary bees are vulnerable.

Foodweb analysis can give insight in what may be the consequences of biodiversity loss for ecosystems. In chapter 4 the effects of biodiversity on the frequency of interactions between flowering plants and flower visiting insects were described in such a foodweb context. The research question was: What is the effect of biodiversity on (1) connectance (the proportion of all possible interactions between plants and insects that are actually established), (2) the number of insect species that visit a plant species ("plant linkage level"), and (3) on the number of plant species an insect species visits ("insect linkage level")? The effects were analysed with path analysis.

The mean connectance per census was $0.21 \pm 0.01$ SE, the mode 0.33. Plant species richness was the most important predictor in the path model. The mean plant linkage level per census was $2.27 \pm 0.09$ SE and the mean linkage level was $35.0 \pm 3.9$ SE. The mean insect linkage level per transect was $1.29 \pm 0.02$ and the mean total linkage level was $15.9 \pm 1.3$. Plant species richness was the most important predictor for connectance. There was a negative direct and positive indirect effect of plant diversity on the number of insect species that visit a plant species: on average, plants were visited by less insect species when there were more plant species. But there were more visitor species per plant species when insect species richness was higher. Insect species richness in turn was positively affected by plant species richness.

Insect linkage level varied much less, and the effect of plant species richness was positive: the more flower species are present in a vegetation, the more species an average insect species will visit. An important finding was that the abundance of a species (plants and insects) increased the chance for the number of interactions with other species.
Although this may sound rather trivial, it is often forgotten or doubted in literature. Furthermore, the effects varied in strength and direction between taxonomical and functional groups of plants and insects. In conclusion, human-induced differences in species richness of plants and insects do affect plant-flower-visitor networks. In literature so far the effect of biodiversity on connectance and linkage levels were only observed across natural biodiversity ranges at larger geographical and temporal scales.

**Pollination and seed set of individual plant species**

In the next three chapters the focus is on plant species rather than the entire community. The effects on pollination of plant diversity (chapter 5), insect diversity (chapter 6), and of diversity in relation to plant population size (chapter 7) are studied.

In chapter 5 the effects of a plant species’ neighbouring plants on its visitation and pollination were analysed with six plant species from the large field data set, and a small garden experiment with Scabiosa columbaria. Contrary to chapter 4, the type and abundance of other flowering species in a community rather than plant species richness affected the number of visiting insect species per plant species (field data). Plants that are more similar interact more with each other. Analysing data of individual and only of generalist species may be the cause of this difference with chapter 4.

In the experiment with S. columbaria the effects of plant neighbourhood on visitation rate and pollination were analysed with patches of potted Scabiosa plants. In patches with high surrounding plant diversity, visitation was highest, but pollination quality lower than in a patch with a less diverse plant neighbourhood. Thus flower visitation rate alone may be misleading when one wants to evaluate facilitation or competition between plants; the resulting pollination and reproduction may be different and even opposite. The main conclusion from this chapter is that the balance between facilitation and competition between plants depends on plant population size and flower abundance of both the target and other plant species in the community, and on plant community species composition.

Chapter 6 is about the vulnerability to pollinator species loss of plant species with various degrees of specialisation. In other words, are pollinators equally exchangeable when biodiversity decreases for different plant species? And how important is insect pollination compared to self-pollination? A field experiment was conducted with generalist plants with generalist visitors (Anthriscus sylvestris and Succisa pratensis), specialist plants with generalist visitors (Phyteuma spicatum subsp. nigrum and Scrophularia nodosa), and specialist plants with specialist visitors (Campanula rotundifolia and Lysimachia vulgaris). Seed set of the plant species with potential self-pollination capacity was considerably reduced without insect visitation, indicating that self-pollination is a less secure solution for pollinator deficiency than is often assumed. For very common and extremely generalist plant species (here: Anthriscus), there will always be some visitors present acting as pollinators. The two specialist plant species with few specialist pollinators were the most vulnerable to pollinator loss: for Campanula and Lysimachia the absence of their oligolectic bees had a negative effect on visitation rate, seed set and germination. For the remaining generalist and specialist plant species with generalist pollinators, predictions are difficult to make.
In chapter 7 the role of biodiversity for pollination is placed in a context of population characteristics and individual pollinator behaviour. The effects of plant and insect species richness and population size on the individual behaviour of insect visitors, insect visitation, pollen deposition and purity of deposited pollen were analysed for *Succisa pratensis*. Large syrphids (hoverflies) and bumblebees were by far the most important pollinators. Large populations of *S. pratensis* were visited by more insect species than small. Visitation rates did not differ between *S. pratensis* populations, but pollination quality and quantity were lowest in small pollinations. The size of *S. pratensis* populations was much more important than diversity of flowering plants and flower visiting insects. The individual behaviour of insects (flower constancy measured directly and indirectly by analysing pollen loads on insect bodies and the pollen deposited on stigmas of the flowers) was affected by population size, explaining the differences in pollination.

*Andrena marginata*, a specialist bee foraging on *S. pratensis*, is extinct in the Netherlands. If it was a better pollinator than syrphids and bumblebees, then the possible shift to a more generalist pollinator assemblage of syrphids and bumblebees had negative consequences for the pollination of *S. pratensis*. The abundance or absence of specialist pollinators can be good indicators of declining plant population sizes, because insects will react faster than plants.

**Minimal insect diversity for the pollination of a plant community**

During the field work for this thesis a few abundant insect species were repeatedly observed on many plant species. In chapter 8 this was addressed quantitatively: Are most plant species in a community visited and pollinated by a small set of dominant insect species? The data set of the transect observations was used for analysing this. Indeed, only ten insect species (2.5% of the species observed!) were very often observed on most plant species; these insects constituted more than 50% of the visitors of two thirds of the analysed plant species. But if all plant species should get 50% and 75% of their visitors, respectively 39 and 93 of the observed insect species were needed. For a guaranteed pollination of the entire plant community the top-ten species are not enough. Firstly, some of the "top-ten" visitors are low quality pollinators. Secondly, more pollinator species are needed for the pollination of the plant community for reasons involving the degree of specialisation of plants and pollinators, variation in space and time, plant population size and density, and interactions between plants. For a sustainable minimal pollinator fauna, a good deal of habitat heterogeneity, continuous food supply throughout the flowering season and a diverse vegetation are needed. In summary, insect species that seem to be "important" as pollinators due to their frequencies (chapters 2-4), may appear to be less important or even negligible when the number of visits (the visitation rate) or other pollination qualities are considered.

**Conclusions**

The answer to the question about the importance of biodiversity for pollination differed between ecological levels: at plant community level, a high pollinator diversity can be important for the pollination of all plant species (chapters 2-4 and 8), while an individual
plant species will often only need its particular pollinators (chapters 5-7). One important aspect, the individual behaviour of insects, got relatively little attention and was only directly measured in chapter 7. The behaviour of species does not necessarily give a clue about the individual behaviour. Scoring only frequencies can be misleading as an estimate of pollination quality, and visitation speed (the number of flowers an insect visits in a sequence) can provide more information. Similarly, the number of plant species an insect species visits (insect linkage level) do not tell how flower-constant an individual is. Frequencies are observations of single moments in time, while individual behaviour shows what happens in a continuous time period. The individual behaviour differs between species and even within species between individuals, partially depending on plant community composition.

**Biodiversity and pollination: Plant species level**

The importance of the diversity of flowering plants and flower-visiting insects for the reproduction and maintenance of a plant species depends on the context: the composition of the flower neighbourhood of the target species, the plant's pollination biology and life history (chapter 6), the degree of specialisation (chapters 4 & 6) and plant population characteristics, i.e. size, isolation and density (chapters 6 & 7). The type of hypothesis about the role of biodiversity for pollination of individual plant species or populations must be of the "context-dependence" type. Predicting the vulnerability of single plant species and populations to loss of biodiversity, particularly pollinators, can only be done by combining species- and site-specific data.

**Biodiversity and pollination: Plant community level**

The diversity of plants and insects is important for a guaranteed long-term pollination of an entire plant community. Many insect species are needed for the pollination, and these insects require a diverse plant community (chapter 8). However, this is not for all flower-visiting insects equally the case. For plant-pollinator communities it is more important that trophic interactions such as pollination take place, rather than which exact species is eating or pollinating which other species. This means that the functional diversity is more important than species diversity for species interactions and diversity relations. Thus for a plant community various insect groups, including generalist and specialists, need to be present. The continuous availability of a variety of various flower types is needed for maintaining a diverse pollinator assemblage. Also here a minimal level of functional (flower) types is required. Exceptional are strict specialists that will need their specific host or partner (e.g. monolectic bees, butterfly larvae and specialist plants). It seems likely that the role of biodiversity lies somewhere between all species and some keystone-species. A proposal for a name for such a hypothesis is "critical diversity level hypothesis".

**Pollination, management and conservation**

Mowing of road verges and ditch banks is a normal management measure. This has also been applied in the study sites of this thesis. Analysing the effect of the management (mowing) of road verges and ditch banks on pollination was not the goal of this thesis, but
due to its frequent occurrence it could not be ignored. Data about the effects of mowing on flowering and flower visitation were shown in chapter 3 and box 9.1. The management and mowing regimes were extremely variable, and the frequency varied between 0-6 times per site (box 9.1). Some parts of the research area where several verges were mown within the same period could be without any flowers for a week or more.

At sites in continuous agricultural areas often no alternative food sources in the time after mowing were available for the insects, as there were no flowers in the crop fields or intensively used grasslands. The effect of no alternative food sources was apparent for bees: the number of solitary bee species and individuals was significantly higher in unmown or mown sites with unmown patches or meadows in the vicinity, compared to mown sites without alternative foraging possibilities (chapter 3). The majority of the plant species could produce new flowers after mowing, but (much) less than before (box 9.1). The flower-visiting assemblage and the amount of visitation before and after mowing differed only for generalist plant species, but not for specialist plant species. In what way this may affect pollination and the reproductive output of a delayed or second flowering cannot be said from the data in this thesis.

In agricultural landscapes the direct effects from mowing, and indirect effects from land use, such as spraying and fertiliser input, should be tackled together. The type of management is crucial for the conservation of plants and animals. Which type of management is chosen depends on the conservation goals and other functions of a habitat. However, in some parts of northern Drenthe any management goals other than keeping the vegetation as short as possible seem to be lacking. Conservation in rural areas requires a better coordination and cooperation than currently is the case.

For the conservation of plants, particularly in fragmented habitats, the habitat requirements of pollinators and the phenology of both flowering plants and their pollinators are important criteria. Concerning mowing, plant and insect phenology are important criteria. If hay is not removed within two weeks after mowing or not at all, accumulation of nutrients can lead to plant species-poor vegetation types. A high plant diversity and flower availability are necessary flower visiting insects. For the plant communities studied, these insects are several groups of flies, syrphids, short- and long-tongued bumblebees, and polylectic and oligolectic solitary bees. Rare plant species may indirectly depend on common species, therefore also core and common plant species should be integrated in conservation. Increasing habitat heterogeneity in agricultural areas will have a positive effect on insect species richness. These insects are needed for pollination of wild plants, and several crops. A higher insect diversity also increases the chance for insects that can control pests and thereby contribute to biological pest control.