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Effects of nitrogen deposition on nutritional quality of producers and its subsequent effects on consumers

VBNE

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for ecosystem restoration

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Foto voorkant	**Top left:** Gryllus campestris in a laboratory feeding experiment using plant material from a full factorial phosphate and lime addition field experiment (photo Joost Vogels). **Bottom right:** First stage instar of *Hipparchia statilinus* on the host plant *Corynephorus canescens*. This species overwinters in this stage, enabling the larva to feed on high quality fresh leaves in early spring on this grass species of general poor nutritional value. Also note the oversized head portion, enabling the organism to chew on the tough *Corynephorus* leaves (photo Marijn Nijssen). **Top right:** Lycaenid butterflies drinking from a mud pool. This is a well-known behavior for nectar-feeding insects in order to acquire additional elemental nutrients which are deficient in floral nectar resources (photo Michiel Wallis de Vries). **Bottom left:** This bull is forced to graze in a nutrient poor heathland environment. Physiological study of these grazers evidenced P-deficiency to occur, probably stimulating these herbivores to show this unorthodox feeding behavior (photo Michiel Wallis de Vries).
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Voorwoord

Vanaf het midden van de vorige eeuw zijn de emissies en depositie van reactief stikstof in de vorm van stikstofoxiden (NO_x) en in gereduceerde vorm (ammoniak NH_3) eerst sterk toegenomen en sinds 1990 ook weer sterk afgenomen. De depositie van stikstof is echter nog steeds één van de grootste 'drukfactoren' die de kwaliteit van (stikstof gevoelige) habitattypen en soorten bepaald. De ecologische impact van de te hoge N-depositie is in Nederland uitzonderlijk hoog. Voor het behoud en bescherming van natuurkwaliteit in Nederland is kennis over de ecologische effecten van de N-depositie van groot belang.

Veel studies naar de effecten van N-depositie op ecosystemen zijn gericht zich op de impact op bodem- en waterchemie, productiviteit en plantengemeenschappen. De effecten op de consumenten van planten (herbivoren en de hogere trofische niveaus) richten zich op enerzijds indirecte effecten (bijv. verruiging, veranderingen in microklimaat, afname nestgelegenheid) en anderzijds op chemische veranderingen (veranderingen in plantkwaliteit). Een groot kennishiaat ligt in de effecten van N-depositie op de chemische samenstelling van planten (producenten) en de consequenties daarvan op de consumenten.

In dit rapport wordt uitvoerig aandacht besteed aan dit specifieke aspect. In de ecologie wordt vanuit verschillende invalshoeken onderzoek gedaan naar variatie in voedselkwaliteit en het effect ervan op het functioneren van consumenten. Daarom is in dit rapport (in de eerste 2 delen) zo veel mogelijk beschikbare relevante literatuur over dit onderwerp bijeen gebracht en de effecten van stikstofdepositie geanalyseerd.

Geconcludeerd kan worden dat het aantal specifieke aan N-depositie gerelateerde studies naar de relatie tussen producenten en consumenten te laag is en bestaan er belangrijke kennishiaten om verstrekkende conclusies te trekken over algemeen geldende mechanismen, de richting en/of de effectgrootte van N-depositie op de producent-consument relaties.

Op basis van de in de literatuur beschreven mechanismen zijn in meer algemenere zin wel uitspraken te doen over de belangrijkste en/of waarschijnlijke causale verbanden. Het laatste deel van dit rapport (deel 3) richt zich dan ook op het identificeren en inschatten van de gevoeligheid van in Nederland voorkomende Natura 2000 Habitattypen en geassocieerde diersoorten voor het optreden van veranderingen in voedselkwaliteit als gevolg van N-depositie. Deze inschatting berust op dit moment voor een groot deel op aannames.

Soorten die relatief nauwe niches in meso- tot oligotrofe, slecht gebufferde tot zure habitats bezetten, lopen de grootste kans om negatieve effecten te ondervinden van de door chronisch verhoogde N-depositie veranderde voedselkwaliteit. Juist voor door Natura2000 beschermde habitats en daaraan geassocieerde soorten heeft deze conclusie grote implicaties, aangezien veel van deze habitats en soorten aan deze omschrijving voldoen.

Kennisverbreding en verdieping is nodig om een adequaat N emissie reductiebeleid vorm te geven en om maatregelen te ontwerpen die deze nadelige effecten kunnen mitigeren: één onderzoeksveld is **de voor voedselkwaliteit belangrijkste plant chemische processen die door N-depositie veranderen en d**e veranderde producent-consument interacties én een tweede on**derzoeksveld** dat uitgaat van het herstel van **nutriëntenbalansen**, **(micro)nutriëntgehalten en antivraatstoffen** (in tegenstelling tot een herstel dat puur is gefocust op het creëren van N-arme omstandigheden) om maatregelen te ontwikkelen die de van natuurlijke processen weer (gedeeltelijk) kunnen herstellen.

Ik wens u veel leesplezier.

Teo Wams
Voorzitter van het OBN Platform/Adviescommissie OBN

OBN Ontwikkeling en Beheer Natuurkwaliteit

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Part 1 – Review outline and general nutritional mechanisms

1 Introduction and methods

1.1 Global increase of reactive nitrogen

Deposition of reactive nitrogen (hereafter referred to as N) from the atmosphere has increased dramatically since the beginning of the 20th century, resulting from combustion processes (e.g. Vitousek et al. 1997), but most notably due to the invention of chemical N fixation, which currently is exceeding natural annual N fixation (Vitousek et al. 1997, Galloway and Cowling 2002, Erisman et al. 2008, Elser 2011). Globally, rates of N deposition have increased strongly since around 1950 (Smith et al. 1999, Galloway and Cowling 2002, Holtgrieve et al. 2011), with current dry deposition hotspots situated in Northwest Europe, Eastern United States, and Eastern China (Jia et al. 2016; **Figure 1**). Due to global warming, wet N deposition is expected to be exacerbated in Eastern United States, India, China, Southeast Asia and Northwest Europe as a result of changes in annual precipitation (Sinha et al. 2017).
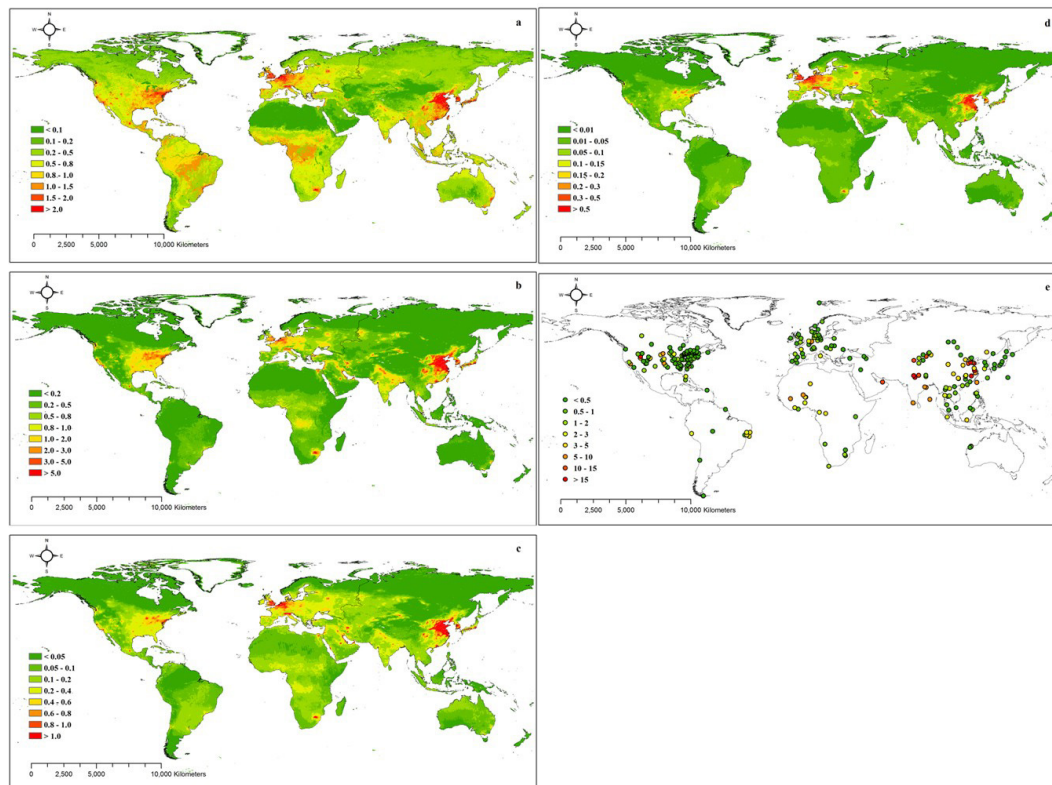
Figure 1. Global differences in mean dry deposition of N (kg N ha^{-1} a^{-1}) for (a) NO$_2$, (b) HNO$_3$, (c) NH$_4^+$, (d) NO$_3^-$ and (e) NH$_3$ for the time period 2005-2014. Figure from Jia et al. (2016). Note that these maps only depicts dry deposition of N.

Figuur 1. Werelwijde verschillen in gemiddelde droge depositie van N (kg H ha^{-1} j^{-1}) voor (a) NO$_2$, (b) HNO$_3$ (c) NH$_4^+$, (d) NO$_3^-$ en (e) NH$_3$ voor de periode 20015-2014. Figuur uit Jia et al. (2016). Merk op dat deze kaarten enkel droge depositie weergeven.

This dramatic increase of reactive nitrogen into the biosphere has major implications for ecosystem functioning, most notably in those that are naturally limited by N and include all major (terrestrial, freshwater, riverine, estuarine and marine) biomes (Ryther and Dunstan 1971, Smith et al. 1999, Elser et al. 2009b, Bobbink et al. 2010b). Total annual N deposition in the Netherlands has been

and continues to be very high, but does show considerable variation on a local scale (**Figure 2**), which is mainly a result of on the distribution and local concentration of emission sources. This is the reason why there is a great sense of urgency to study and address the issue of excessive N deposition in the Netherlands.
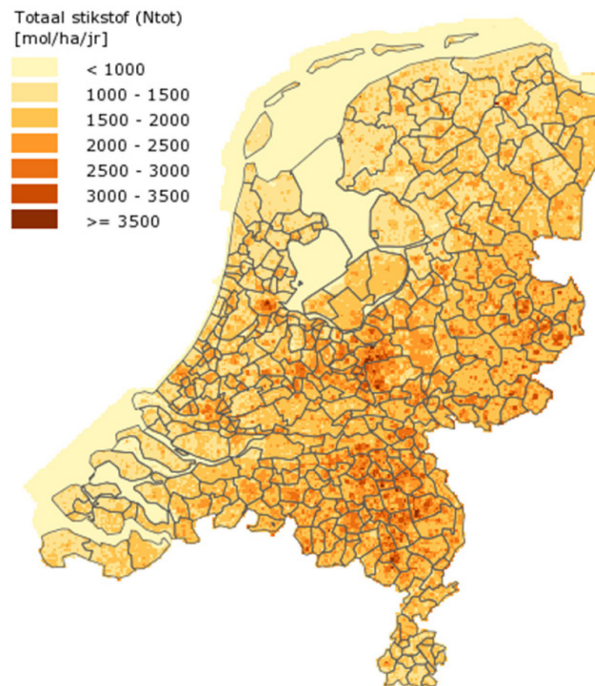
Figure 2. *Nationwide differences in calculated total annual N deposition (mol·ha^{-1}·year^{-1}) in the Netherlands in 2018 (RIVM 2020); https://www.rivm.nl/gcn-gdn-kaarten.*

Figuur 2. *Nationale verschillen in berekende totale jaarlijkse N-depositie (mol·ha^{-1}·year^{-1}) in Nederland in 2018 (RIVM 2020); https://www.rivm.nl/gcn-gdn-kaarten.*

1.2 Scope of this report

Several studies have reported effects of increased N deposition on primary production (e.g. Stevens et al. 2015), soil and water chemistry (e.g. van Breemen et al. 1984, Houdijk 1993, Hogg et al. 1995, De Graaf et al. 1998) and plant communities (e.g. Bobbink et al. 1998, Bobbink et al. 2010b, Stevens et al. 2010, Stevens et al. 2011, Field et al. 2014). Studies that focus on the effects of N on higher trophic levels, or on the response of specific consumer species to N deposition are more scarce, but also are receiving increased interest during the last decade. Nijssen et al. (2017) provided an overview of several pathways (**Figure 3**) that either directly or indirectly influence fauna performance, which include N deposition driven changes in quantitative (food plant quantity, prey availability), physical (microclimate, reproductive habitat) and chemical (food plant quality, chemical stressors) aspects of habitat conditions. One of the major knowledge gaps addressed in this review article are the effects of changed producer chemistry on consumer performance. In this report, we aim to provide more insight in this specific aspect by reviewing relevant literature regarding producer-consumer interactions, N-deposition induced changes in soil, water and plant chemistry and specific studies aimed at the effects of such induced changes on consumer performance.

By applying the insights and hypotheses obtained from this literature review, we then conducted a preliminary test of hypotheses on predicted causal mechanisms affecting species response (either positive or negative) and vulnerability in case of reduced food quality, using a trend analysis of different butterfly species differing in life history and nutritional niche between regions differing in long-term N deposition. In addition, we constructed a sensitivity analysis of all existing terrestrial

and freshwater N2000 protected habitat types in the Netherlands and associated fauna species to N deposition induced changes in food quality.
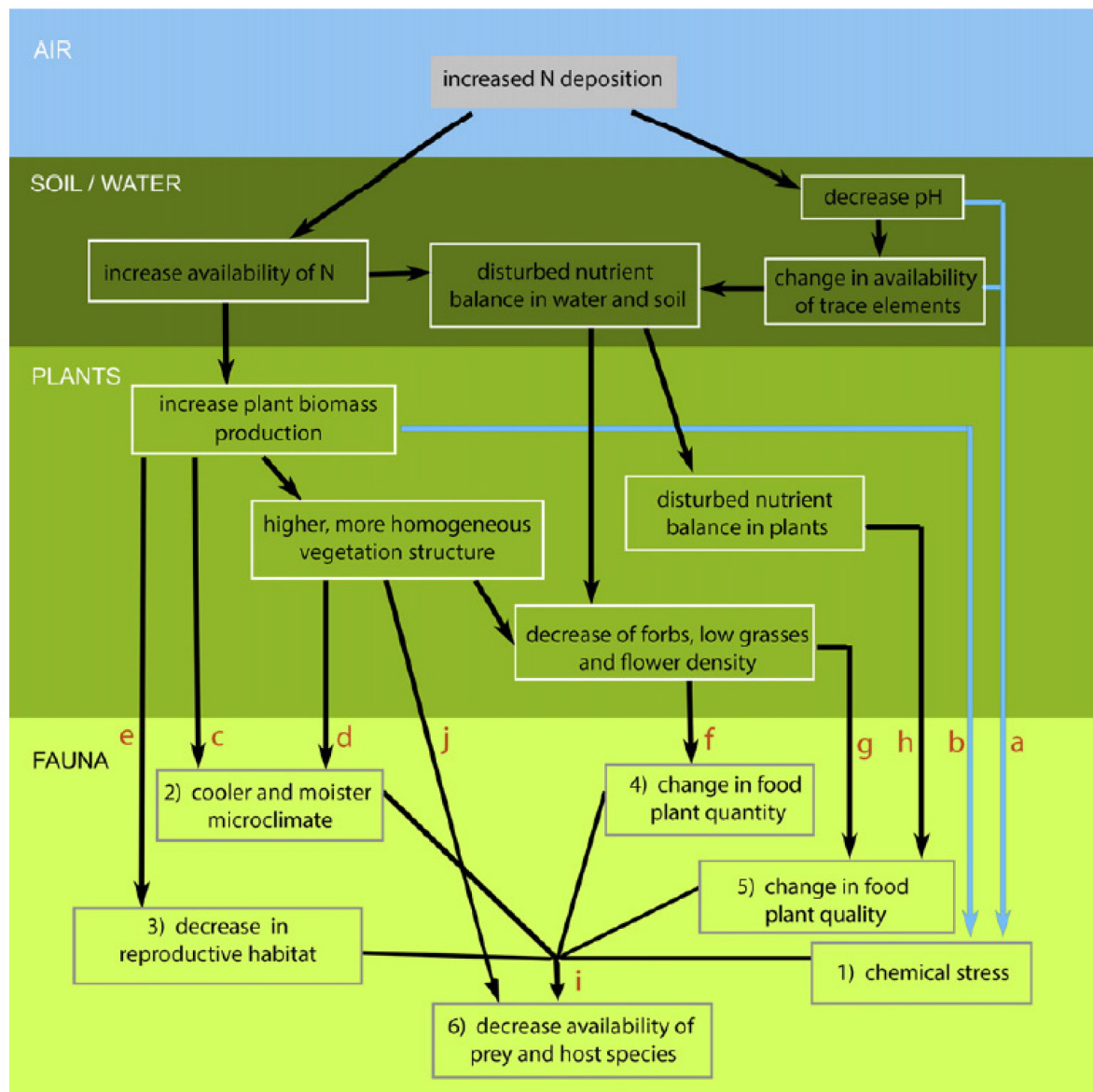
Figure 3*. Pathways of direct and indirect effects of N deposition on fauna. Figure from Nijssen et al. (2017). In this report, we will mainly focus on pathway g and h, which can be summarized as "changes in food plant quality affecting consumer fitness," which will include a review on all underlying mechanisms and pathways in soil and autotroph organisms. Cascading effects through higher order trophic levels (pathway i), and when relevant in this context, chemical stress inducing pathways (a, b) have been covered as well.*

Figuur 3*. Causale routes van directe en indirecte effecten van N-depositie op fauna. Figuur uit Nijssen et al. (2017). In dit rapport zullen we ons voornamelijk richten op route g en h, die kan worden samengevat als 'veranderingen in de kwaliteit van voedselplanten die de fitness van de consument beïnvloeden', waar we een overzicht geven van alle onderliggende mechanismen en routes in de bodem en autotrofe organismen. Doorwerkende effecten naar hogere trofische niveaus (route i), en indien relevant in deze context, routes die chemische stress veroorzaken (a, b) worden eveneens behandeld.*

1.2.1 Research questions

The main research question following from this scope can be formulated as follows:

"How does nitrogen deposition affect the nutritional quality of food plants and what are the subsequent effects on animal species?".

In this study higher plants as well as algae are treated as food plants, and from now on referred to as 'producers', and all animal species as 'consumers'. This main question is obviously much too broadly formulated. In this review we therefore attempted to provide answers to the following questions:

1. What are the chemical aspects of producers that influence the nutritional value for consumers? In other words: "how can producer nutritional quality be defined?"
2. How are consumers adapted to naturally occurring inadequacies in nutritional quality of producers?
3. Which ecological traits or foraging strategies influence consumer species response to N deposition driven changes in producer quality? *Which traits or strategies are likely to lead to increased performance, which to reduced performance, which reduce the likelihood of significant effects?*
4. Under which field conditions does N deposition lead to significant changes in producer nutritional quality? *More specifically; how are these N deposition driven changes related to geology, soil processes, hydrology, soil buffering capacity etc.?*
5. Which producer nutritional quality parameters are known or highly likely to be affected by N deposition and/or acidification?
6. Are there specific cases in peer reviewed literature that report N deposition mediated changes on consumer performance and if so, what nutritional aspect, species interactions and ecological strategies were investigated?
7. Which habitat types, target species and/or ecological functional groups of consumer species are vulnerable for N deposition induced changes in producer quality?
8. Which questions cannot be answered with present data or knowledge regarding the occurrence of bottlenecks in producer quality caused by nitrogen deposition?

Note that these questions are not addressed in chronological order in the report (see below for the structure of the report).

1.3 Reader's guide

Given the complexity and broad scope of this review report, we decided to divide this report into four major parts. The **first part** consists of the introductory paragraphs, the scope of this review report, followed by a review of the major nutritional frameworks used in ecology. Especially these frameworks occupy a substantial amount of this part (and even this report), and we are aware that at first glance this section does not always provide an immediate or obvious link with the effects of N deposition on consumer performance. The reason why this part has been worked out to such degree is that we feel that it is essential to start with a good understanding on the mechanisms that exist naturally in consumers in dealing with (naturally occurring) inadequate food quality.

Why this is so important is twofold: first, evolution is a driver of the specific nutritional needs of different organisms, and second, this nutritional need itself is also an evolutionary driver shaping the specific behavioral and physiological adaptations of different organisms in coping with inadequate food quality. Since there are many different nutritional needs and even more nutritional strategies, this implies that the consequence of changed nutritional quality due to increased N deposition largely depends on the 'nutritional niche' a given organism occupies, and the specific ecological strategy that it uses to cope with the naturally occurring environmental conditions. As a result, the effects of N deposition on consumer performance can differ considerably between species, up to the degree of a diametrical response of two species that at first glance are adapted to the same habitat conditions and that may be found regularly in the same habitat. Therefore, a thorough understanding of the current knowledge in the field or nutritional frameworks is essential to predict, understand or interpret real-world shifts in community composition, species declines or

increases, plague events, etc., all in the context of changed nutritional quality driven by N deposition.

In this overview of nutritional frameworks, we do however include the major findings concerning N deposition mediated changes reported in literature and perhaps more importantly, devise hypotheses about the implications of increased N deposition on producer-consumer interactions with respect to the framework under review. A summary of these can be found at the end of each subchapter under 'summary conclusions'. This part of the report finishes with a resumé and general conclusion chapter, in which we summarize the major findings and hypotheses resulting from this review part in the context of increased N deposition.

The **second part** of this report first reviews the current knowledge on the effects of increased N deposition on ecosystem functioning and consumer performance. It starts with a (brief) description of N deposition effects on soil and water chemistry, and then reviews the relevant literature of N deposition effects on producer quality as well as current knowledge regarding the effects of such (N deposition driven) changes on first and higher order consumers. This part consist thus mainly of N deposition specific studies and results of N enrichment experiments and is thus much more directly linked to N deposition effects on producer quality and its subsequent effect on consumers. We followed the same structure and order as used in the first part, using the nutritional frameworks as starting point, starting from the most simple (CNP stoichiometry) to the most complex (micronutrients) framework.

The knowledge reviewed and summarized in the first part is often used in order to put the results our outcomes of the experiments in a broader context. Not surprisingly, the amount of existing literature that specifically address N deposition effects on producer quality and consumer response is inversely correlated with this degree of complexity. Maybe as important as the review of existing literature and knowledge obtained from these is thus the identification of specific knowledge gaps concerning specific producer-consumer interactions, especially those that are have been hypothesized to be of significant importance in the first part of this report.

We finalize this second part of the report with a partial test of hypotheses obtained in the nutrient frameworks and specific N deposition effects chapters. We do this by comparing the trends of butterfly populations in the Pleistocene part of the Netherlands, subdividing between regions differing in long-term N deposition. We use species nutritional strategies (i.e. monophagous vs generalist species, grass vs herb vs woody host plants, etc.) and the 'trophic niche width' (i.e. occurring in a broad range of habitats differing in nutrient status vs species restricted to specific meso to oligotrophic environments) as predictors influencing he sensitivity to changed nutrient quality due to N deposition. We finalize this part again with a resumé and general conclusion chapter, in the same manner as for the first part.

The **third part** of the report consists of an estimation of the sensitivity of specific N2000 habitat types and associated species for increased N deposition, with respect to changed food quality. The estimation will be made using the knowledge of ecosystem and functioning and trophic effects reviewed in chapters 1 through 5, when available. Do note, however, that for many habitat types and even more species, there is a considerable lack of knowledge on the exact ecological mechanisms and thus the existence and/or severity of impact can often only be hypothesized at best. What this part does provide is a first estimation of the likelihood that N deposition mediated shifts in nutrient quality can occur in a given N2000 habitat in the Netherlands, and the likelihood that a given associated species of N2000 habitats is vulnerable to such changes in nutrient quality, given the current state of knowledge.

We stress that such a list cannot be used as a matrix that delineates the degree of vulnerability of ecosystems for N deposition driven changes in nutrient quality. In other words; it is not a list of factual knowledge, but a mixture of experimental evidence, correlative studies, reported cases and even hypotheses only. Its usefulness is that it may act as a helpful tool to prioritize measures to reduce N-emissions (cases that are based on experimental evidence), to prioritize the development of mediating measures (based on the knowledge reviewed here) and to prioritize further research

on specific ecosystems and/or species vulnerability (those hypothesized to be vulnerable but are yet lacking in sufficient evidence).

The **fourth and final part** of the report consists of a report summary prioritized research agenda, **written in Dutch**. The research agenda is founded on all aspects reviewed and investigated in this report and can be summarized as an answer to the following question: "when applying the results and hypothesized effects of N deposition mediated changes in nutrient quality, which species, habitat types and/or food quality aspects deserves further investigation in order to gain insight in the mechanisms, direction and severity of impact and subsequent implications for the biodiversity and community composition of animal species that are dependent on N2000 habitat types?" We chose to write this part in the Dutch language as this research agenda has a high research focus on research on N deposition effects on nutritional food aspects as it (might) occur in The Netherlands.

1.4 Glossary

In this report, terms are used of which the meaning or our intended demarcation of its meaning is not always clear to every reader. In order to improve the readability of the report, we here provide a glossary table (**Table 1**), including abbreviation terms and a short explanation of the terms used.

Table 1. *Glossary table of technical terms and/or often used abbreviations in the report.*

Tabel 1. *Verklarende woordenlijst met technische termen en/of vaak gebruikte afkortingen die in het rapport gebruikt zijn.*

Term	Abbreviaton	Explanation
Acid Neutralizing Capacity	ANC	Acid-neutralizing capacity or ANC in short is a measure for the overall buffering capacity against acidification for the soil or a surface water.
Allelochemical		Compounds produced by autotroph organisms, that often play a role in plants defence to consumers. A wide variety of compounds exist differing in elemental composition and structure. Includes antifeedants and phytotoxins (for explanation see corresponding glossary terms)
Antifeedant		Chemical compound produced by autotrophs used as plant defence mechanism, functions via impairment of nutrient uptake efficiency of the consumer
Base Saturation	BS	Base saturation expresses the percentage of the CEC of the soil occupied by the base cations Ca^{2+}, Mg^{2+}, K^+ and Na^+, taking into account the corresponding charge units of the ions.
Cation Exchange Capacity	CEC	Term used in soil biochemistry, being one of the factors determining the soil acid neutralizing (ANC) capacity. Cation exchange capacity is defined as the amount of cations that can be exchanged per mass or volume of soil (molc/kg, formerly meq/kg). CEC is measured in moles of electric charge, so a cation exchange capacity of 10 molc/kg could hold 10 mol of K^+ (with molc 1 unit of charge per mol cation) per kilogram of soil, but only 5 mol Ca^{2+} (2 molc units of charge per mol cation).
Compensatory feeding		Behavioral response mechanism in consumers: when offered food of low nutritional quality, ingestion rate is increased considerably in order to obtain adequate amounts of macronutrients.
Complementary feeding		Behavioral response mechanism in consumers: when first offered food of imbalanced (macro)nutritional quality (e.g. too low protein:carbohydrate ratio), ingestion of complementary food items (i.e. having a high protein:carbohydrate ratio) is actively increased contra the suboptimal food item, in order to match the optimal intake ratio of macronutrients as close as possible.
Docosahexaenoic acid	DHA	Essential omega 3 type fatty acid with 6 unstaturated carbon bonds

Table 1. *(continued)*

Term	Abbreviaton	Explanation
Ecological stoichiometry	ES	Field of study that investigates the effect of elemental nutrient ratios (with an emphasis on the macro-elements C, N and P) on ecosystem functioning, producer-consumer interactions and species performance
Essential (micro)nutrient		Organic nutrient that cannot be synthesized by a given consumer organism and thus has to be obtained by consuming adequate amounts in the food. Includes specific amino acids, vitamins and fatty acids. Set of essential (micro)nutrients differs between consumer organisms depending on metabolic physiology of the organism.
Holobiont		An assemblage of a host and all other species living in or around it, which together form a discrete ecological unit. Here mostly referred to as the symbiosis of the host and all relevant endosymbionts aiding in the acquirement of nutrients for the host.
Highly unsaturated fatty acid	HUFA	Fatty acids with 3-6 unsaturated carbon bonds
Intake target		Used in NG theory: the absolute amount as well as the ratio of protein and carbohydrate at which maximum consumer performance is achieved.
Macronutrient		Organic molecules primarily made out of C, N (and P) for consumers that are required in large quantities for an organism to function.
Macro-element		One of the three major elements that make up most of the biomass of organisms: C, N and P
Micronutrient		Nutrient that is essential in organismal functioning, but is only required in low quantities in the food.
Nutritional Geometry	NG	Field of study that focuses on the response of consumers to absolute macronutrient availability as well as the macronutrient ratio in food. Used in identifying (behavioral) response mechanisms in consumers in optimizing growth under differing (often suboptimal) macronutrient availabilities and/or ratios.
Non-proteionomic amino acid		Amino acids not used in protein synthesis that can be metabolized by producers. May act as phytotoxins.
Non-protein nitrogen	NPN	Nitrogen in producers not incorporated in proteins. May be present in producers in the form of free amino acids, simple organic molecules, or non-proteionomic amino acids.
Osteophagy		The behavior of consuming bone tissue in order to obtain high quantities of Ca and/or P. Mainly evidenced in vertebrates, especially ungulates.
Phytotoxin		Chemical compound produced by autotrophs used as plant defence mechanism, functions via disruption of consumers metabolic, neurological or physiological functioning.
Poly unsaturated fatty acid	PUFA	Fatty acids with 2 unsaturated carbon bonds
Threshold Elemental Ratio	TER	Used in ES theory: the optimal C:N, N:P or C:P ratio at which maximum consumer performance is achieved.

2 Nutritional frameworks in ecology

2.1 A variety of food quality definitions

Primary producers take up elements and inorganic compounds from the abiotic environment, and convert these into organic molecules. Optimal conditions for producers involve ample availability of light, macro-elements (notably N and P), and micro-elements (e.g. trace elements), as well as sufficient water (for terrestrial producers) and inorganic carbon (for aquatic producers). These resources are assimilated into biochemical compounds, such as carbohydrates, fatty acids, lipids, amino acids, proteins, and nucleic acids, as well as a range of secondary metabolites. Many of those act as important nutrients for consumers. The elemental and biochemical make-up of primary producers can thus be considered as the key determinant of food quality for consumers.

Various definitions of food quality used in ecological literature involve concentrations of specific nutrients in producers, ranging from single elements (e.g. N, P, Na, Ca) to molecules differing in the degree of complexity (e.g. macronutrient composition vs. essential amino-acid composition), interactions (single nutrient vs. nutrient ratios), or focusing on secondary metabolites that hamper nutrient uptake (antifeedants and toxins). Although attempts have been made to integrate the different research frameworks (most notably between ecological stoichiometry and nutritional geometry theory; Raubenheimer and Simpson 2004, Sperfeld et al. 2017), many studies to this date use one definition of this set of nutritional frameworks as the (proximate) factor determining food quality. Also, most of the research fields covered do not solely focus on the impact of altered nutritional quality on consumers, but often aim to better understand broader ecological concepts, such as ecosystem functioning, trophic interactions, energy flow and ecosystem resilience.

In this chapter, we review the existing nutritional frameworks in ecology without specifically addressing the impact of increased N deposition on the functioning of the mechanisms involved in these frameworks, and summarize the findings in the light of N deposition at the end of each framework addressed. The knowledge summarized here thus provides a mechanistic understanding of the processes behind N deposition effects on nutritional functioning, which will be addressed in part 2 of this report.

2.2 C:N:P stoichiometry

The research field of ecological stoichiometry (Reiners 1986, Sterner and Elser 2002, Hessen et al. 2013) studies the balance of elements in ecological interactions from cells to ecosystems, and as such involves the effects of macronutrient ratio's (C:N, N:P, C:P ratios) on producer-consumer interactions. In this field, nutrient quality is measured as the degree of similarity between producer and consumer C:N:P stoichiometry. As heterotrophs are more C:N:P homeostatic than autotrophs (Kagata and Ohgushi 2006, Persson et al. 2010), the degree of limitation by a nutrient is also dependent on its relative availability to other nutrients. In other words, elemental imbalances of primary producers, relative to more homeostatic consumers, may hamper consumer performance and thereby lower their fitness.

2.2.1 C:N:P stoichiometry in producers

The overall stoichiometry and elemental demands of primary producers is determined by their biochemical make-up. Biochemical compounds consist of distinct sets of elements (**Table 2**). For instance, amino acids, and thereby proteins, contain relatively high amounts of N. Nucleic acids, and thereby RNA and DNA, are relatively rich in N but especially P as compared to other cellular compounds, while carbohydrates, fatty acids and a range of lipids are C-based and lacking N and P.

Some exceptions exist, for instance, phospholipids are P containing lipids used for cellular membranes and P storage molecules (**Table 2**).

Table 2. Relative contribution (by mass) of carbon, nitrogen and phosphorus, and associated the atomic C:N and N:P ratios (by mole) of several key biochemicals (Van de Waal and Boersma 2012).

Tabel 2. Relatieve bijdrage (massa%) van koolstof, stikstof en fosfor, en geassocieerde molaire C:N en N:P ratios van verscheidene belangrijke biochemische componenten (Van de Waal and Boersma 2012).

		%C	%N	%P	C:N	N:P
Carbohydrates		37	0	0	-	-
Fatty acids	Butyric acid (C4:0)	55	0	0	-	-
	Lignoceric acid (C24:0)	78	0	0	-	-
Amino acids	Tyrosine	60	8	0	8.7	-
	Arginine	41	32	0	1.5	-
Nucleotides	DNA[a]	33	15	8.7	2.6	4.0
	RNA[a]	30	14	8.4	2.4	3.8
Lipids	Triacylglycerols[b]	75	0	0	-	-
	Phospholipids[c]	65	1.6	4.2	48	0.9
Proteins	Average[d]	47	15	0	3.6	-

[a]*Average of the four bases present in the respective nucleic acids;* [b]*Representing a typical triacylglycerol (tripalmitin);* [c]*Average of five important phospholipids (phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidyldiglycerol, sphingomyelin; Sterner and Elser 2002);* [d]*Average of all 20 proteinogenic amino acids.*

Primary producers can exhibit a high stoichiometric plasticity as carbon and nutrients are acquired via separate pathways and requirements can be down-regulated, e.g. through reduced protein levels (Geider and La Roche 2002, Liefer et al. 2019). At the same time, non-limiting elements may be effectively accumulated in storage molecules, such as phospholipids and certain amino acid based storage polymers (Geider and La Roche 2002, Sterner and Elser 2002, Elser et al. 2010a). Together, reduced levels of a limiting nutrient and accumulation of a non-limiting nutrient can cause significant changes in primary producers C:N:P stoichiometry (Gonzalez et al. 2017, Garcia et al. 2018). Indeed, although Redfield (1958) found that the N:P ratio of marine phytoplankton in the surface ocean closely matched that of dissolved in organic N:P availabilities in the ocean interior (at a molar ratio of 16:1), the stoichiometry of stoichiometry of individual species or along spatial gradients can be more variable. For instance, C:N:P ratios were shown to change along a latitudinal gradient (Martiny et al. 2013). In freshwater lakes and terrestrial ecosystems, mean C:N:P ratios deviate from the Redfield ratios and furthermore show higher variability (Elser et al. 2000a, Sterner et al. 2008; **Figure 4**).

Variation in primarily producer N:P ratios are largely determined by the availably of inorganic N and P in the environment, where N limitation will lead to lower N:P ratios and P limitation to higher N:P ratios. Both N and P limitation by primary producers were shown to be common in terrestrial, freshwater and marine ecosystems (Elser et al. 2007), which may at least partly explain the natural variation in N:P ratios reported for autotrophs (**Figure 4**). Such variations in primary producer stoichiometry may have consequences for higher trophic levels.

2.2.2 C:N:P stoichiometry in consumers

Ecologists have long recognized the influence of plant elemental quality on herbivore survival, growth rate, reproduction, consumption, and population dynamics (Liebig 1841, Lindeman 1942, Redfield 1958, Mattson 1980, Reiners 1986, Mattson and Scriber 1987, White 1993, Sterner and Elser 2002, Hillebrand et al. 2009). The available evidence reviewed here however rests mainly on studies involving invertebrate herbivores. Initially the role of energy (carbon) and energy flow through food webs received most attention (Lindeman 1942), but also the importance of nitrogen (N) availability in many plant-animal interactions has for long been broadly recognized (Mattson 1980). White (1993) argued that the availability of energy was much less often limiting growth and reproductive success of herbivore populations, and that nitrogen availability was most often the

limiting factor. According to this author, N availability is the major restricting element for all animals, dictating consumer performance and related ecosystem processes. Thus, both C and N may be limiting factors potentially restricting consumer performance, and their relative availabilities in the food may thus determine, at least partly, which factor is limiting consumers.

Generally, primary producer C:N ratio is much higher and much more dynamic than herbivore C:N ratio (Fagan et al. 2002, Sterner and Elser 2002, Hessen 2008, Sardans et al. 2012b, Hessen et al. 2013). Indeed, tissue N content is often found limiting herbivore performance, reproduction or density (Hessen 1992, Throop and Lerdau 2004, Throop 2005, Kagata and Ohgushi 2006, 2007, Couture et al. 2010, Loaiza et al. 2011, Lebigre et al. 2018). This is especially the case in species which contain high amounts of N such as grasshoppers (Fagan et al. 2002), a species group where N-limitation has often been demonstrated in field and laboratory experiments (Loaiza et al. 2008, 2011). For aquatic systems, typically the consequences of P limitation on herbivore performance have been studied intensively (Sterner and Elser 2002, Hessen et al. 2013), while few studies tested the role of N on herbivore performance. For example, a higher relative N content in algae (i.e. a lower C:N ratio) was shown to exhibit higher growth rates and egg production rates of a marine copepod as compared to food containing a relatively low N content (i.e. high C:N ratios) (Kiørboe 1989, Burian et al. 2018).

Although N limitation in plant-herbivore interactions may indeed be widespread, this does not mean that it is the limiting nutrient under every circumstance. Indeed, several authors reported a negative (Fischer and Fiedler 2000, Kay et al. 2007, Zehnder and Hunter 2009, Nijssen and Siepel 2010, Cease et al. 2012, Pöyry et al. 2017, Zhu et al. 2019) or a quadratic (Joern and Behmer 1997, 1998) herbivore response to higher tissue N content.
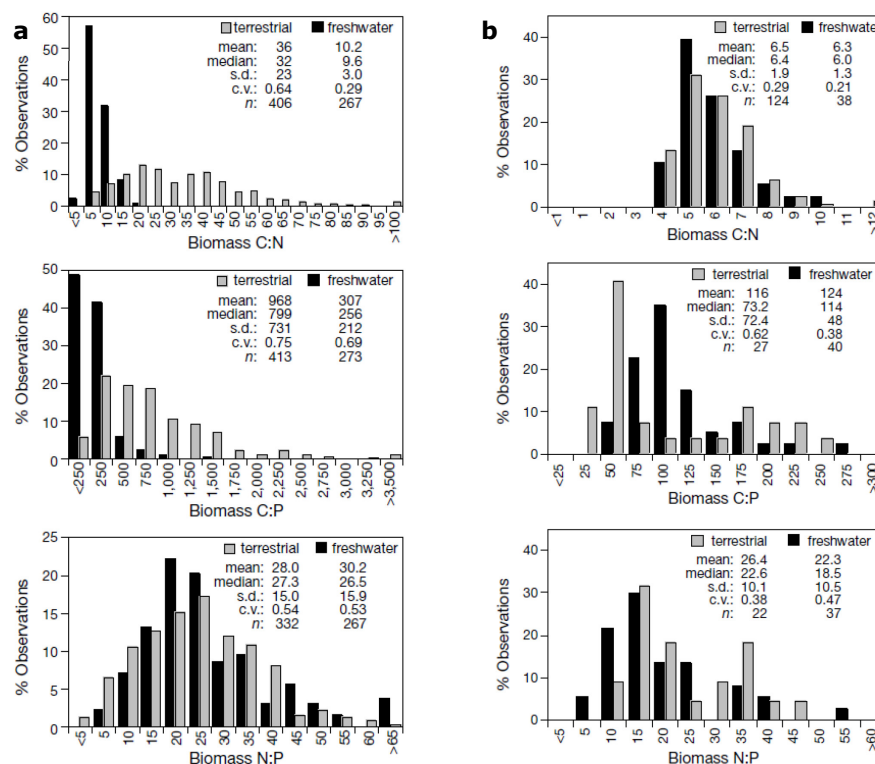
Figure 4*. Frequency histograms summarizing atomic C:N:P stoichiometry of autotrophs (a) and invertebrate herbivores (b) in terrestrial and freshwater habitats. Data and figures from Elser et al. (2000a).*

Figuur 4*. Frequentie histogrammen die de (molaire) C:N:P stoichiometrie van autotrofen (a) en ongewervelde heterotrofen (b) in terrestrische en zoetwater habitats samenvatten. Data en figuren uit Elser et al. (2000a).*

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Elser et al. (2000a) summarized autotroph and invertebrate herbivore C:N, C:P and N:P ratios and concluded that in both freshwater and terrestrial ecosystems, next to inadequate N intake, consumers can encounter P-deficiency. Indeed, the relative deficiency for P is often higher than for N, as autotrophs have a significantly higher mean N:P ratios than herbivores (**Figure 4**).

This finding was corroborated by Gonzalez et al. (2017) who found no niche overlap in C:N:P stoichiometry between primary producers, invertebrates and vertebrates (**Figure 5**A), herbivores and non-herbivores (detritivores and predators; **Figure 5**B). Sardans et al. (2012b) concluded that tissue N:P ratio is more critical than C:N ratio in explaining bottom-up effects through trophic levels and concluded N-limitation by consumers may also reflect N:P co-limitation. The degree of P limitation in herbivores, however, increased in direct proportion to the degree of N limitation, which implies that under increasing N and P limitation (resulting in low leaf tissue N and P), P availability is more likely to become limiting for herbivores. It may thus be appropriate to conclude that the relative availability of C, N and P (as well as other elements such as Na and K) influences consumer performance, and that no single element can be pointed out as the single limiting nutrient for consumers.

Also very important to note is that under increasing availability of one of the nutrients, increased growth of producers can have dilutive effects on the concentration of the other elements, hereby reducing the relative availability of the element that is non-limiting for producers. Such a nutrient dilution effect has recently attributed to be a causal factor in the gradual decline of a North American grasshopper (Welti et al. 2020), in that particular case through dilution of food N, P, K and Na via increased biomass production resulting from increased CO_2 concentrations (i.e. effectively increasing C:N, C:P, C:K and C:Na concentrations of producer tissue).
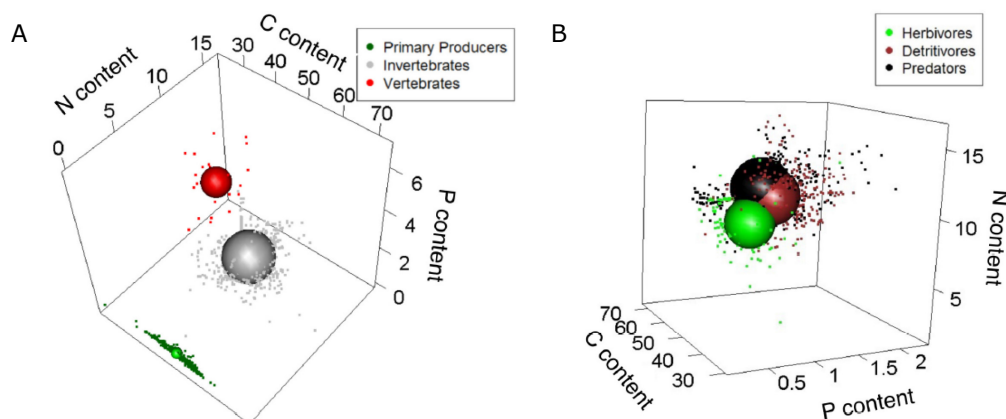
Figure 5. Stoichiometric niche space and volume of A) primary producers, invertebrates and vertebrates, and B) herbivores, detritivores and predators (figure from Gonzalez et al. 2017). Sphere sizes depict volume of stoichiometric niches, centered around the average C, N, and P contents of all individuals. Axes represent each element as a percentage of dry body mass.

Figuur 5. Stoichiometrische nicheruimte en volume van A) primaire producenten, ongewervelde dieren en gewervelde dieren, en B) herbivoren, detritivoren en predatoren (figuur uit Gonzalez et al. 2017). Grootte van de bolvormen geven het volume van stoichiometrische niches weer, gecentreerd rond de gemiddelde C-, N- en P-gehalte van alle individuen. Assen vertegenwoordigen elk element als percentage van de droge biomassa.

2.2.2.1 Threshold Elemental Ratio
As indicated in the previous paragraph, higher relative nutrient contents in primary producers (i.e. lower C:P or C:N ratios) not necessarily lead to higher food quality with enhanced grazing performance and/or fitness. In insects for instance, a high N content of the food was shown to reduce performance (measured as population growth rate, survival rate, RGR, development time, adult body mass; Zehnder and Hunter 2009, Cease et al. 2012). Comparably, animal performance (Mass gain rate, RGR) was shown to decrease at the higher range of dietary P content, presumably due to energetic and metabolic costs invested in excreting excess nutrients (Boersma and Elser

2006). Thus, for any given nutrient ratio, there often exists an optimal ratio at which maximum performance is achieved. By introducing the Threshold Elemental Ratio (TER; Urabe and Watanabe 1992) in ecological stoichiometry theory, quadratic relationships between producer macronutrient contents and consumer performance are explicitly taken into account. The TER is defined as the ratio between elements A and B at which nutrient A is equally limiting as nutrient B. Following TER, a reduction in the A:B ratio would lead to limitation of A, while an increase in the A:B ratio would lead to a limitation by B (e.g. **Figure 6**). Animals feeding on food with nutrient ratios at the TER exhibit maximum growth at given nutrient ratio (Sterner and Elser 2002). The TER thus equates to the optimum C:N; C:P and/or N:P ratio for growth, and can be considered the elemental analogue to the 'intake target' (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993) in the nutritional geometry framework (Sperfeld et al. 2017) (see also section 2.4.3).

The TER of different organisms can vary widely as a result of the organism's life history, ontogeny, trophic status, mobility and growth rate. Such interrelationships between different elements provide an explanation for contrasting or level dependent species responses to dietary nutrient content manipulation experiments (e.g. found by Joern and Behmer (1998), Huberty and Denno (2006), Audusseau et al. (2015), and Lebigre et al. (2018)).
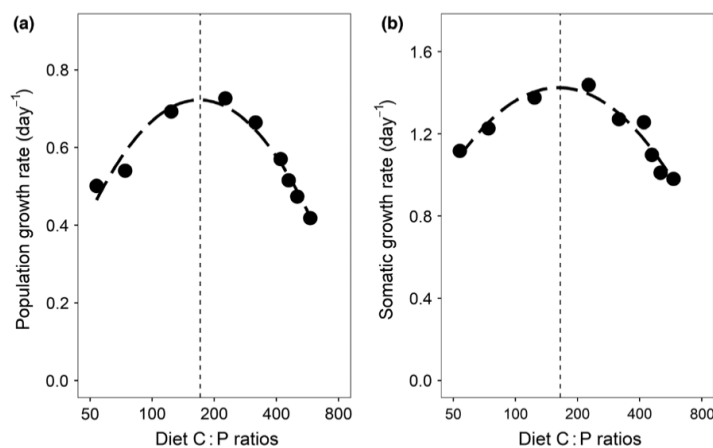
Figure 6. *Examples TER for C:P ratios in algal food for rotifers (from Zhou and Declerck 2019).*

Figuur 6. Voorbeelden van TER voor C:P ratios in uit algen bestaand voedsel voor Rotifera (uit Zhou and Declerck 2019).

2.2.2.2 Differences in C:N:P stoichiometry between aquatic and terrestrial habitats
Various studies have compared C:N:P stoichiometry of producers and consumers in aquatic and terrestrial habitats. Early work by (Elser et al. 2000a) for instance showed that for primary consumers, the producer-consumer difference in N:P ratio was comparable between aquatic and terrestrial habitats. Based on these findings, the authors concluded that the degree of limitation should be comparable between aquatic and terrestrial habitats. These comparisons were, however, based on overall means and variations in unrelated producer and -consumer elemental ratios.

More recently, Lemoine et al. (2014) compared C:N:P ratios of producer-consumer pairs in a terrestrial, stream and lake ecosystem, and concluded that potential N-limitation was strongest in the stream, intermediate in terrestrial, and lowest in the lake ecosystem. Potential P-limitation was also strongest in the stream ecosystem, and equally strong in the terrestrial and lake ecosystem, but in the lake ecosystem, the magnitude of P-limitation was greater than for N, which makes it more likely for herbivores in lake ecosystems to experience P-limited growth than in terrestrial systems. This study thus suggests that there are differences in the strength of macronutrient limitation between aquatic and terrestrial habitats.

Whether enhanced N deposition will cause an increase in herbivore performance, however, will depend on the producers responses. When strongly N limited, for example in streams or terrestrial

ecosystems, N deposition may, at least partially alleviate limitation and thereby be beneficial for herbivores. In freshwater systems where P is more often limiting, additional N may only have small consequences. Yet, we note that, as also indicated earlier, there may be large spatial and temporal variations in the extend of N and P limitation, particularly in more eutrophic systems that exhibit strong feed-backs from primary producer growth.

2.2.2.3 N:P Threshold elemental ratio in terrestrial herbivores

Studies that find significant positive effects of P-addition or correlations of dietary P include many species, life stages and feeding strategies, including Lepidoptera larvae (Goverde et al. 2000, Perkins et al. 2004, Apple et al. 2009), grasshoppers (Bishop et al. 2010, Ibanez et al. 2017), reproducing female or displaying male crickets (Bertram et al. 2009, Visanuvimol and Bertram 2010, 2011), curculionid beetles (Schade et al. 2003), mesophyll-feeding lacebugs (Kay et al. 2007), honeybees Filipiak et al. (2017) and phloem feeding planthopper species (Huberty and Denno 2006).

However, there are also many studies that find no positive effect of higher or experimentally increased dietary P content (Zehnder and Hunter 2009, Loaiza et al. 2011, Tao and Hunter 2012, Harrison et al. 2014, Zhang et al. 2014) or even negative effects of increased plant P content (Loaiza et al. 2008, Tao and Hunter 2012) on herbivore performance.
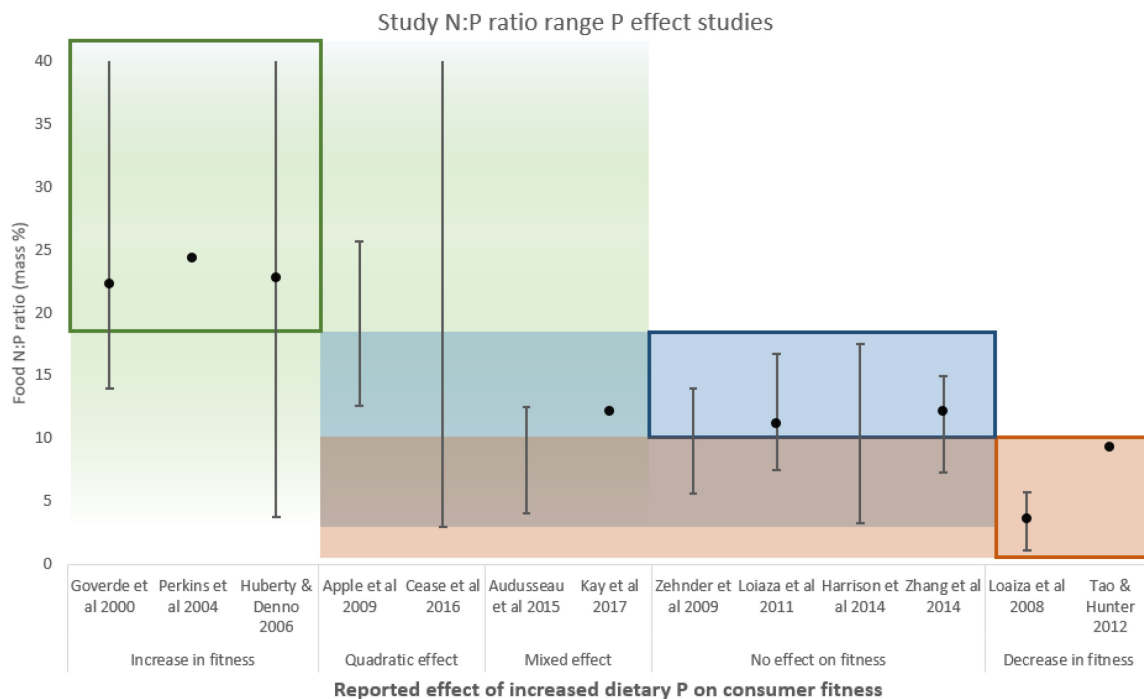
Figure 7. Mean (dots, when retrievable) and range (whiskers, when retrievable) of dietary N:P ratio employed in herbivore fitness effect studies. Data derived from Goverde et al. (2000), Perkins et al. (2004), Huberty and Denno (2006), Kay et al. (2007), Loaiza et al. (2008), Apple et al. (2009), Zehnder and Hunter (2009), Loaiza et al. (2011), Tao and Hunter (2012), Harrison et al. (2014), Zhang et al. (2014), Audusseau et al. (2015), Cease et al. (2016). Orange area denotes (expected) fitness decrease, blue area no effect range and the green area shows (expected) increase in fitness range. Colored encircled rectangles indicate reported positive, no or negative effect outcomes.

Figuur 7. Gemiddelde (punten, indien voorhanden) en bereik (lijnen, indien voorhanden) van de N:P verhouding van het voedsel die gebruikt werd in studies naar fitness effecten bij herbivoren. Gegevens afkomstig van Goverde et al. (2000), Perkins et al. (2004), Huberty en Denno (2006), Kay et al. (2007), Loaiza et al. (2008), Apple et al. (2009), Zehnder en Hunter (2009), Loaiza et al. (2011), Tao en Hunter (2012), Harrison et al. (2014), Zhang et al. (2014), Audusseau et al. (2015) en Cease et al. (2016). Oranje gebied indiceert de (verwachte) afname van fitness, blauw gebied geen effect en het groene gebied indiceert (verwachte) toename van fitness. Gekleurde omcirkelde rechthoeken geven de gerapporteerde positieve, geen of negatieve effectresultaten.

These differences in study outcome might partly be explained by range differences in the elemental ratios used between these studies, or by different nutrient requirements of the investigated invertebrate species, resulting in different species specific TERs and hence different responses to P addition (Audusseau et al. 2015).

Many studies find a quadratic relationship between nutrient content and insect performance (Clancy and King 1993, Apple et al. 2009, Cease et al. 2016) and studies that find reduced performance under higher tissue P (Loaiza et al. 2008, Tao and Hunter 2012) typically investigated plants with relatively low median N:P and/or low N:P range (**Figure 7**, **Table 3**). For instance, mean consumer N:P ratio in Tao and Hunter (2012) matched or even exceeded mean producer N:P ratio. TER theory can therefore provide an explanation for the direction of response to relative shifts in increased dietary N:P found in dietary manipulation studies.

2.2.2.4 *Physiological and phylogenetic determinants of consumer N:P TER*
As indicated in the previous paragraph, the degree and the direction of which an organism responds to a shift in producer C:N:P (i.e. the location of it's TER) is dependent on an organisms physiology and ontogeny, both of which are possibly also delimited by phylogenetic constraints. This is especially relevant for the determination of the N:P TER in consumers, which will be discussed in this section. In animal cells and body parts, the most common N-rich biomolecules are proteins, making up major fractions in the biomass of organs and muscles (Elser et al. 1996). The most P-rich biomolecules are DNA and, particularly, RNA in ribosomes. In vertebrates, also bone tissue in the form of the bone mineral apatite ($Ca_5(OH)(PO_4)_3$) greatly contributes to overall P demands (Elser et al. 1996). Thus, vertebrates as well as organisms and/or life stages or organisms that have relatively high amounts of ribosomes will also exhibit high demands for P.

Since growth, and subsequently protein synthesis, mainly occurs during this life stage, larval stages of insects typically contain more P than the adults (**Table 3**). More generally, invertebrates with higher growth rates are also shown to have higher P requirements for ribosomes needed for enzyme synthesis (Elser et al. 1996, Elser et al. 2000b). High RNA content and RNA:DNA ratios have often been found to be positively correlated with the organisms relative growth rate (Elser et al. 2003, Watts et al. 2006, Van Geest et al. 2010, Sardans et al. 2012b). Furthermore, as growth rate is allometrically negative related with size, smaller species have relatively higher P demands as compared to larger species. This pattern holds up until larger body size can only be possible with a supporting endoskeleton, which is rich in P (Elser et al. 1996). This Growth Rate Hypothesis (GRH) has found experimental supported in invertebrates in aquatic ecosystems, but for terrestrial ecosystems evidence is not yet conclusive (Sardans et al. 2012b) and might be dependent on other factors, such as climatic conditions (Hambäck et al. 2009). Woods et al. (2004) however found an inverse relationship between body mass and phosphorus content in insect and arachnid species and Watts et al. (2006) found a negative relationship with larval growth rates and body C:P and N:P ratios in *Drosophila melanogaster*, suggesting that for terrestrial organisms the GRH has predictive value as well.

Body functions that are linked to high protein synthesis, such as male signaling (Bertram et al. 2006) and egg production (Visanuvimol and Bertram 2010) have also found to be positively correlated with body P content. Organisms or life stages with a high growth rate should either eat relatively P-rich food or implement physiological adaptations focused on maximizing P-extraction, P-retention and exhibit overall high P use efficiencies (Woods et al. 2002, Zhang et al. 2014). Besides physiological and life-history trait variation, relative N and P requirements have also been linked to evolutionary patterns. Tissue N content is lower in more recently evolved terrestrial insect orders (Fagan et al. 2002) and this pattern is also partially present for tissue P content (Woods et al. 2004), suggesting that continuous evolutionary pressure results in a continuous selection pressure focused on minimalizing N and P needs (**Figure 8**).

Table 3. *Summary table of methodical setup, reported range and/or mean C:N:P content and ratio (mass balance) of producers and consumers and results of studies on effects of differing dietary C:N:P ratios on insect fitness and growth.*

Tabel 3. *Overzichtstabel van methodische opzet, gerapporteerd bereik en / of gemiddeld C:N:P-gehalte en verhouding (massa%) van producenten en consumenten en resultaten van studies naar effecten van verschillende C: N: P-verhoudingen in de voeding op de fitness en groei van insecten.*

Study	Species	Life stage	Food type	C%	N%	P%	C:P ratio	C:N ratio	N:P ratio	Effect
Goverde et al. (2000)	*Polyommatus icarus*[1]	larva to adult	*Lotus corniculatus* on AMF and non AMF treatment	40.5[a]-43.4[b]	40.5[a]-43.4[b]	0.13[a]-0.37[b]	321[a]-118[b]	6.9[a]-8.26[b]	46.3[a]-14.3[b]	Increased mortality and reduced growth in non-AMF fed larvae.
Schade et al. (2003)	*Sibinia setosa*[2]		natural experiment with field collected *Prosopis* leaves and weevils				200-1200			Weevil body P%, % RNA and abundance negatively correlated with plant C:P
Perkins et al. (2004)	*Manduca sexta*[1]	Egg to terminal instar	Manipulated natural food plants; artificial diets			0.21-1.18	243[c] vs 62[d]		24.4[c] vs 15.3[d]	Increasing diet P significantly increased growth rate, body P and decreased time to final instar moult.
Huberty and Denno (2006)	*Prokelisia dolus* (flightless) and *P. marginata* (flying)[3]	Adult	Fertilized food plants *Spartina alterniflora*		1.1-7.8	0.08-0.68			3.7-97.5 (mean 22.8)	Flying *P. marginata* strongest affected by lowered plant N and to lesser extent lower P.
Kay et al. (2007)	*Corythuca arcuata*[3]	Eggs and adults	Burned and unburned *Quercus macrocarpa*		2.18-2.71	0.18-0.22			12.3-12.11	No effect on lacebug density
Loaiza et al. (2008)	*Melanoplus bivitattus*[5]	5th instar nymphs	Artificial diets differing in protein:carbohydrate ratio and P content					11-22	1.1-5.7	Slower growth at high P diet (N:P=1.1-1.8)
Apple et al. (2009)	Lepidoptera on *Lupinus lepidus*	larvae	*Lupinus lepidus* in field and greenhouse	44-47	1.4-2.9			15.9-32.9 (mean 22.3)		Grazing lowers plant N content, promoting insect performance and stimulating locust outbreaks
Zehnder and Hunter (2009)	*Aphis nerii*[4] on *Asclepias syriaca*	nymph to adult	*Asclepias syriaca* grown on differing N and P levels		3-6	0.3-0.9		5.6-14		No effect of P on growth, quadratic effect of N on growth. Higher P avail led to higher plant N thus slower growth.

Table 3. *(continued)*

Study	Species	Life stage	Food type	C%	N%	P%	C:P ratio	C:N ratio	N:P ratio	Effect
Loaiza et al. (2011)	Grasshopper species[5]	nymphs and adults	Field experiment in tallgrass prairie with N and P fertilization; 'choice experiment' where grasshoppers were able to migrate to plots.		1-1.5[e] 1.35-1.75[f]	0.09-0.14[e] 0.11-0.2[f]			0N: 10.4[e] / 9.6[f] 10N: 13.1[e] / 11.8[f]	Grasshopper densities increased significantly in N fertilized plots, not in P fertilized plots.
Cease et al. (2012)	Oedaleus asiaticus[5]	adults	Naturally growing plants affected by grazing pressure	44-47	1.4-2.9			15.9-32.9 (mean 22.3)		Grazing lowers plant N content, promoting insect performance and stimulating locust outbreaks
Tao and Hunter (2012)	Danaus plexippus[1] and Aphis asclepiadis[4]	larvae, generation	N and P treated milkweed plants in greenhouse		1.3-3.2	0.12-0.64			9.4	No effect of plant P on D. plexippus, negative effect of plant P on A. asclepiadis performance
Harrison et al. (2014)	Gryllus veletis[6]	adults	Artificial diets differing in protein:carbohydrate ratio and P content		3.68	0.45-2.45			3.22-17.5	Protein and carbohydrate ratio determined fitness, nof effect of P levels on performance
Zhang et al. (2014)	Oedaleus asiaticus[5]	Adults	Naturally occurring plants in field cage experiment with different grasshopper density		0.8-1.6	0.08-0.14			12.2[g] vs 16.7[h]	P excretion positively correlated with food plant P, resulting in P homeostasis
Audusseau et al. (2015)	Aglais urticae, Polygona c-album, Aglais io[1]	Egg to terminal instar	N and/or P fertilized field collected Urtica dioica						4-12.5	Two species respond positively to N fertilization, one to P fertilization
Cease et al. (2016)	Schistocerca americana[5]	3-5th instar nymphs	Artificial diets varying in P%		4.46	0.02-1.50			3.0-223	Increased growth and survival with higher P, with optimum P of 0.25-0.50. High P reduced growth and survival.

[1] Lepidoptera; [2] Coleoptera: Curculionidae; [3] Hemiptera: Auchennorrhyncha; [4] Hemiptera: Sternorrhyncha; [5]Orthoptera: Acrididae; [6] Orthoptera: Gryllidae.
[a]Non-AMF inoculated; [b]AMF-inoculated; [c] Naturally occurring plants; [d] Larval *Manduca sexta*; [e] *Andropogon*; [f] *Solidago* ; [g] Food plant; [h] Grasshopper.

For the most recently derived Lepidoptera and Diptera however, this pattern is consistent for N but not for P (Woods et al. 2004) and tissue N:P ratio of these orders is therefore generally substantially lower than older lineages, resulting in stronger linkage between host plant P nutrition and species performance (see par.2.2.2.6). Within Diptera, phylogeny accounted for a large fraction of variation in N content, but not so much for P content (Hambäck et al. 2009).
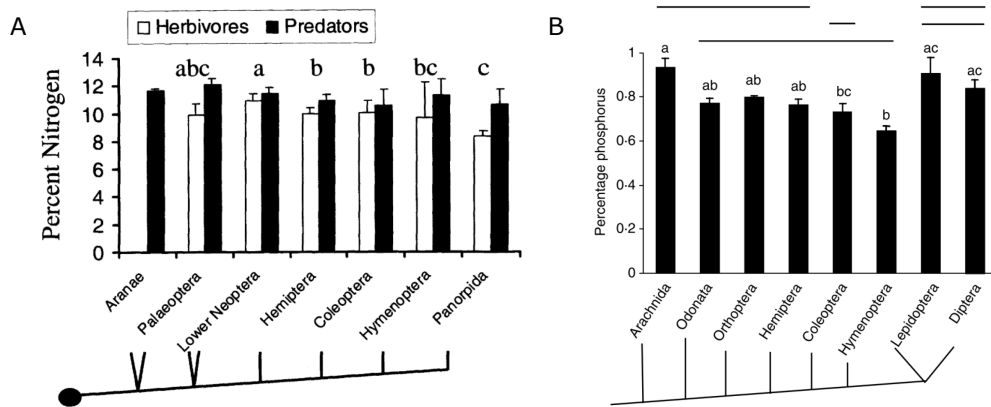
Figure 8. *Mean body tissue (± 1 S.E.) nitrogen (A) and phosphorus (B) content of invertebrate taxa, grouped phylogenetic relationship. Figures taken from Fagan et al. (2002) for N and Woods et al. (2004) for P. For figure B mean P content did not differ significantly between predators and herbivores and both trophic groups are plotted combined over the taxonomic lineages. Note that the Lepidoptera and Diptera in figure B are combined as Panorpida in figure A.*

Figuur 8. *Gemiddeld (± 1 S.E.) stikstof (A) en fosfor (B) gehalte van ongewervelde taxa, gegroepeerd op basis van fylogenetische verwantschap. Figuren uit Fagan et al. (2002) voor N en Woods et al. (2004) voor P. Voor figuur B verschilde het gemiddelde P-gehalte niet significant tussen predatoren en herbivoren en beide trofische groepen zijn tezamen weergegeven over de taxonomische groepen. Merk op dat de Lepidoptera en Diptera in figuur B zijn gecombineerd als Panorpida in figuur A.*

2.2.2.5 Regulatory mechanisms in C:N:P balancing

Since stoichiometric mismatches between producers and consumers for the all three major macro-elements C, N and P exist in the natural environment, heterotrophic organisms have employed many behavioral and physiological adaptations to cope with nutritionally imbalanced food. For instance, they are able to compensate nutrient deficiencies by switching between complementary food items or increase net food ingestion under conditions of poor food quality (Bernays and Bright 1991, Berner et al. 2005). The mechanisms of compensatory feeding for C and N are based on carbohydrate and amino acid concentrations of the food measured by the insect during feeding (Raubenheimer and Simpson 1993) and will be discussed later in this chapter (see section 2.4). In aquatic herbivores, compensatory feeding was also shown to occur under macronutrient limitation, with higher feedings rates when under N or P limitation (Fink and Von Elert 2006, Suzuki-Ohno et al. 2012, Zhou and Declerck 2019).

Under nutrient limitation, producers may accumulate C and thereby comprise a relatively high C content. In order to deal with this potentially excess in C, herbivores have developed a range of physiological adaptations. For instance diet induced thermogenesis, revealed by an exponential increase in body heat production was observed in the spruce budworm, effectively increasing respiration rates and thereby enhancing C losses through increased oxidation under excess dietary C supply (Trier and Mattson 2003). Phloem-feeding aphids feed on high C food and are known to excrete excess C as honeydew, which they also use in sustaining mutualistic relationships with ants (Way 1963), hence further reducing the fitness costs associated with excretion of excess C.

The extend by which consumers have to deal with imbalances in e.g. the C:N ratios will depend on their TER for C:N, or more specifically, the intake target ratio for carbohydrate:protein. The TER or intake target ratio for herbivores is not fixed and may depend on external factors. For instance, predation stress in a grasshopper induced a food preference shift from low C:N to high C:N food,

due to higher metabolic C demands under stressed conditions (Hawlena and Schmitz 2010). Moreover, in aquatic ecosystems, the TER was shown to depend on food quantity, and consequently altering the need for compensatory feeding (Fink and Von Elert 2006, Suzuki-Ohno et al. 2012). For N in insects, excretion comes with relatively high costs compared to C, resulting in a more strict intake regulation for N than for C in herbivores (Behmer 2009). Comparably, high P availabilities in food were shown to come at a cost related to storage or excretion of excess P, as was shown in various aquatic herbivores reducing performance at C:P ratios below their TER (Anderson and Hessen 2005, Boersma and Elser 2006, Persson et al. 2010, Zhou and Declerck 2019).

Most studies on terrestrial herbivores find no evidence for compensatory or complementary feeding behavior for P (**Table 6**), even when fitness was significantly influenced by P intake relative to N intake (Perkins et al. 2004, Visanuvimol and Bertram 2010, Harrison et al. 2014, but see Cease et al. 2016). In contrast, however, various studies on aquatic herbivores revealed the potential for compensatory feeding through higher feeding rates under P limited conditions (Fink and Von Elert 2006, Suzuki-Ohno et al. 2012, Zhou and Declerck 2019). Why compensatory feeding for P seems to be commonplace in aquatic systems and not in terrestrial systems remains an unresolved issue in ecological stoichiometry. A possible mechanism is that in aquatic systems, consumers often use a filter feeding strategy on seston and/or phytoplankton (i.e. unicellular or simple organisms with relatively low structural carbohydrate content as compared to higher plants) whereas terrestrial consumers feed on producers rich in structural carbohydrates (high in cellulose and/or lignin) which limits the maximum intake capacity for these organisms.

The responses to nutrient limitation will largely depend on the extent by which herbivores regulate their stoichiometric homeostasis. For instance, some herbivores, referred to as regulators, exhibit mechanisms to maintain homeostasis at a range of intermediate carbon:nutrient ratios, while breakdown of homeostasis may occur at either lower or higher ratios. In contrast, other herbivores, referred to as conformers, may follow food carbon:nutrient ratios at an intermediate stoichiometry range, while exhibit homeostasis at both extremes (Meunier et al. 2014). The degree of P adsorption by herbivores has been found negatively correlated by plant tissue P content and this has been found to be caused by increased gut P uptake under low plant tissue P concentration (Woods et al. 2002, Zhang et al. 2014). In aquatic ecosystems, *Daphnia* and *Holopedium* respond to increased P limited conditions by increasing alkaline phosphatase enzyme concentrations (Elser et al. 2010b, McCarthy et al. 2010).

2.2.2.6 Species traits, feeding guilds and life history

The sensitivity of consumers to stoichiometric imbalances caused by N deposition will not only depend on the consumer physiology (e.g. sections 2.2.2.2 and 2.2.2.4), but also on a number of consumer traits, including specialist or generalist feeding strategies. For example, Boersma and Elser (2006) propose that consumers specialized on food with a high C:nutrient ratio are more susceptible to deviations in the C:nutrient ratio. As these organisms have evolved living on low nutrient food, their own tissue C:nutrient ratios are often relatively high. Also, oligotrophic environments may also be more stable with respect to inorganic nutrient concentrations. Specialist consumers in such environments may show low investments in efficient processing and excreting of excess nutrients. Furthermore, low P content in producers in such systems does not allow to quickly dilute excess nutrients by increasing growth rate in consumers in these systems.

As specialists on low food N and P content already deal with low nutrient supply, alterations in N or P content of food can also quickly lead to reduced performance. This leads to the hypothesis that specialists consumers of nutrient poor food generally occupy a more narrower niche in terms of producer C:N:P stoichiometry, and therefore are more vulnerable to deviations in plant C:N:P stoichiometry, than generalist consumers. In terms of the TER terminology, this can be visualized as a narrower quadratic curve for specialists compared to generalists (**Figure 6**; **Figure 9**). Experimental support for this hypothesis can be found from nutritional geometry studies (further discussed in section 2.4), where host plant specialist typically employ stronger regulation against

excess N (protein) intake than for C (carbohydrate) compared to generalist herbivores (Simpson et al. 2004, Behmer 2009, Warbrick-Smith et al. 2009).
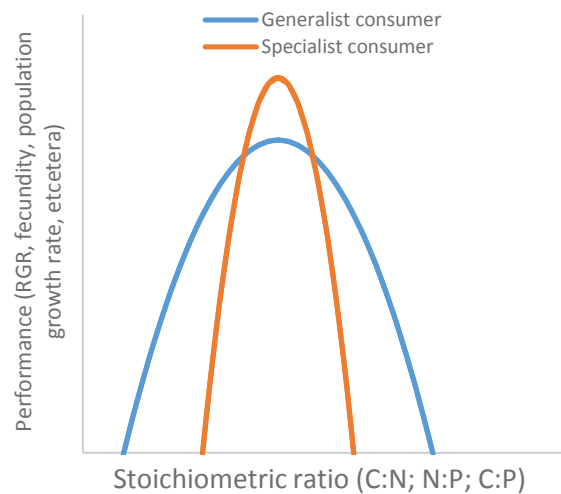
Figure 9. *Visualization of the theoretical response of a specialist vs a generalist consumer to deviations in plant C:N:P stoichiometry. Here, both species have an identical TER, but the specialist speciesis characterized by a narrower fitness curve than the generalist species, and by having a higher absolute fitness at the TER compared to the generalist species.*

Figuur 9. *Visualisatie van de theoretische respons van een specialistische versus een generalistische consument op afwijkingen in plant C:N:P stoichiometrie. Hier hebben beide soorten een identieke TER, maar de gespecialiseerde soort wordt gekenmerkt door een nauwere fitnesscurve dan de generalistische soort, en door een hogere absolute fitness op de TER in vergelijking met de generalistische soort.*

Besides occupying a narrower niche in stoichiometric space, specialist species may also be more adapted to feeding on plants that are closer to their own body tissue C:N:P ratio; i.e. feeding on plants with low C:N and/or N:P ratio. Carnicer et al. (2012) developed a so-called "adaptive trait continuum"; defined as the first axis of a principal component analysis for 169 Mediterranean butterfly species by traits, reflecting univoltine, low dispersal monophagous species on the lower end and multivoltine, high dispersal polyphagous species on the other end of the continuum (Carnicer et al. 2012, Carnicer et al. 2013). In a subsequent study, a negative linear trend in host plant P content and a weak quadratic trend for host plant N content was found over this range (Carnicer et al. 2015). As such, monophagous species tend to feed on plants relatively high in P; hence with low N:P ratio, polyphagous species on plants lower in P% and thus on plants with higher N:P ratio, and the latter group shows a broader range potential in host plant N:P ratio (see Table 4, derived and calculated from Carnicer et al., 2015).

Pöyry et al. (2017) found further supporting evidence on a dataset of European Lepidoptera (including moth species) where larval dietary breath and host plant foliar N was strongly related to adult insect body size. They also found that multivoltine, large polyphagous species eating high N host plants generally show positive population trends during the last decades, in contrast to (smaller) mono-oligophagous species. This suggests that larger, generalist species occupy a relatively high N:element niche (i.e. Low C:N and/or high N:P ratio) and specialist occupying a relatively high P:element niche (i.e. low C:P and N:P ratio). In Diptera, specialist species with a nectar or pollen feeding adult life stage generally have low body N content compared to other Diptera, and P content was inversely related with adult body size (Hambäck et al. 2009). Thus, relatively small flower-visiting Diptera would in general have lower body N:P ratio compared to other Diptera and would hence have a relatively low optimum N:P ratio.

Table 4*. Range of P% and N%, mean N:P ratio (mass%) and regression N:P range of host plant leaf tissue for two contrasting feeding modes of butterflies in the functional trait continuum (Carnicer et al. 2012), as derived from the figure in Carnicer et al. (2015). N% and P% are minimum and maximum values of the partial regression lines for given feeding modes; mean is the mean N:P ratio of these partial regression lines and N:P range is calculated using minimum and maximum N% and P% values in the partial regression lines. Numbers between brackens depict range width.*

Tabel 4*. Bereik van P% en N%, gemiddelde N:P-verhouding (massa%) en uit regressie bepaald N:P-bereik van bladweefsel van de gastheerplant voor twee contrasterende voedselstrategieën van vlinders in het functional trait continuum (Carnicer et al. 2012), afgeleid uit de figuur in Carnicer et al. (2015). N% en P% zijn minimum- en maximumwaarden van de partiële regressielijnen voor de gegeven voedselstrategieën; het gemiddelde is de gemiddelde N: P-verhouding van deze partiële regressielijnen en het N: P-bereik is berekend met behulp van minimum- en maximumwaarden voor N% en P% uit de partiële regressielijnen. Getallen tussen haakjes geven de breedte van het bereik weer.*

	Mono-oligophagous	Polyphagous
P% range	0.17-0.19 (0.02)	0.14-0.17 (0.03)
N% range	2.09-2.29 (0.20)	2.14-2.34 (0.20)
Mean N:P	12.0	14.3
N:P range	11.0-13.5 (2.5)	12.6-16.7 (4.1)

For invertebrate species feeding on whole plants or plant parts, the general rule is thus that larger generalist species are more prone to show N limited growth, and smaller specialist species to show P limited growth, with a narrower N:P ratio bandwidth than generalist species. An exception to this rule is found in consumers that circumvent feeding on whole plant tissue by either feeding on the consumer's nutrient flow on phloem or xylem sap or on cell content rather than whole plant tissue. N:P ratio of phloem sap is often much lower than that of leaf tissue (Tao and Hunter 2012), so phloem feeders should be less limited by P than by N. N-limitation has indeed been found often in studies addressing the response of phloem feeders response to different nutrient levels (Setamou et al. 2016), and in studies that reported negative effects of increased plant P concentration on phloem feeding invertebrates (Tao and Hunter 2012). Lace bugs, who specialize on the cell contents of leaf mesophyll have been reported to respond negatively to high N content and positively to high leaf P content, but only under conditions of high light availability, and Lace bug density was in general more determined by C content of leaves than N or P content. (Kay et al. 2007), suggesting that the optimal C:N intake ratio of this insect was higher than mean oak leave C:N ratio.

In aquatic herbivores, some species, such as copepods, are able to selectively graze on food matching their own nutritional demands, while others, including cladocerans, are generalists (Hessen et al. 2013, Meunier et al. 2016). Copepods have been shown to exhibit generally higher body N:P ratios (and lower body P), and thus seem to have higher N over P demands as compared to cladocerans, with lower body N:P (and higher body P) that seem to favour more P rich food (Andersen and Hessen 1991, Walve and Larsson 1999, Hessen et al. 2013).

2.2.3 Summary conclusions

Based on the literature reviewed in this section, the first conclusion that can be made is that none of the major biological elements (C,N,P), can be regarded as the ubiquitous limiting element for producers or consumers (contra Lindeman 1942, and White 1993). The relative availability of the major elements (i.e. elemental stoichiometry) is the determining factor in assessing individual and/or population growth rate, reproduction and survival, which are optimal at the threshold elemental ratio (TER) of given nutrients. Another general conclusion is that producers show a higher plasticity in elemental stoichiometry than consumers, and generally have higher C:N as well as N:P stoichiometric ratios than consumers. Shifts in producer C:N:P ratios may result from changes in N (and P) availabilities (see also sections 4.2, 0 and 2.2.1).

Under increased N deposition, producer C:N ratios are likely to decrease, but N:P ratios are likely to increase, possibly leading to enhanced or more frequent P limitation. Consequently, consumers

with relatively low N:P TERs are expected to be more often negatively affected by N deposition, as they will become increasingly limited by P at higher N:P ratios. In contrast, species with relatively high N:P TERs are expected to be more often positively affected by N deposition, as food N:P will be more often shifted towards the optimum ratio. The degree and direction of the change in consumer performance resulting from increase N deposition is also dependent on the ecosystem specific C:N:P stoichiometry in producers, as this differs between naturally oligotrophic environments and eutrophic environments. Whether, to what extent and in which direction increased N deposition mediated shifts in producer C:N:P ratios alters the performance of consumers is therefore expected to be ecosystem, producer species well as consumer species dependent. For consumer species, a species specific C:N:P TER is determined by physiological functioning, phylogeny, ontogeny, behavioral adaptations and other species traits such as feeding mode, dispersal capability and host plant and/or food type specialization.

For terrestrial invertebrates, there exists supporting evidence for the hypotheses that 1) specialists occupy a narrower stoichiometric niche than generalists, 2) specialists tend to feed on host plant species that are more similar in nutrient stoichiometry (i.e. in general have a lower N:P ratio), 3) C:N:P stoichiometry in consumers is scaled allometrically (par. 2.2.2.4) and 4) Lepidoptera and Diptera are most vulnerable to changed producer N:P ratio, due to their relatively low body tissue N:P ratio (par. 2.2.2.4) compared to other groups. Under increased N deposition, this may thus lead to a reduction in performance of host plant specialists, which should occur markedly in relatively small, low dispersing univoltine species, and possibly accompanied by an increase in performance of larger, high dispersing generalist species. Such filtering mechanisms on species traits as well as taxonomic lineages should thus ultimately result in a reduction of total biodiversity in a given ecosystem. As phylogenetic constraint is also identified as a determining factor in consumer N and P content, species belonging to taxonomic lineages with relatively low N:P stoichiometry are considered most vulnerable to increased N deposition and associated changes in producer N:P stoichiometry.

These general rules are however greatly modified by the particular ecological niche occupied by the specific organism in question (e.g. terrestrial invertebrates vs aquatic invertebrates), and these hypotheses do not apply to other feeding modes (e.g. grazers vs browsers vs sap suckers vs filter feeders) which all results in marked differences in C:N:P stoichiometry between producers and consumers. Sufficient knowledge on ecosystem functioning, producer response to increased N availability on C:N:P stoichiometry, the consumer species involved and their associated life-history strategies should always be the basis in devising viable hypotheses in Ecological Stoichiometry research in the context of N deposition effects on producer-consumer interactions.

2.3 Other elements and trace metals

2.3.1 The ionome: all other elements making up an organism

The ionome is defined as the mineral nutrient and trace metal composition of an organism, and thus includes all elements other than the major elements C, N and P contained in an organism (Salt et al. 2008, Huang and Salt 2016, Jeyasingh et al. 2017). These elements can be tightly coupled to distinct functions, and therefore support a further understanding on the physiology, evolution and ecology of organisms. These other elements, however, are only recently receiving increasing scientific interest, for instance under the framework of elemental stoichiometry (Jeyasingh et al. 2017, Tian et al. 2019).

2.3.2 Function of elements in producers

Elements such as K, Ca and Mg are required in substantial quantities for cellular and homeostatic functioning, and trace metals such as Iron (Fe), Zinc (Zn), Manganese (Mn), Nickel (Ni), Copper (Cu) and Cobalt (Co) play key roles in various key cellular processes, thus potentially influencing growth of primary producers (**Table 5**; Twining and Baines, 2013). In the marine cyanobacterium *Synechococcus*, for example, a close coupling was reported between Zn and P contents, as well as

between Ni and N contents, which follows fr   om the use of Zn and Ni in enzymes involved in acquiring organic P and N sources, respectively (Twining et al. 2010).

Terrestrial plants vary considerably in elemental composition. Herbs have usually much higher levels of P, K, Ca and Mg than woody plants. In woody plants, deciduous trees have higher levels of these elements than evergreen trees. During the growing season, elemental composition of leaf tissues also substantially changes, with in general higher concentrations of N, P and K at bud break, followed by a rapid decline of these elements over the growing season. Ca, Mg, Mn and Al concentration often steadily increases over the growing season (Mattson and Scriber 1987). Ionomic interactions between producers and consumers are thus dependent on species identity, seasonal changes in elemental composition in producers and spatial differences in elemental composition within the ecosystem.

Table 5. *Common metalloproteins within marine phytoplankton (table taken from Twining and Baines, 2013).*

Tabel 5. *Veel voorkomende metalloproteïnen in marien fytoplankton (tabel uit Twining en Baines, 2013).*

Metal	Protein(s)	Function(s)
Fe	Cytochromes	Electron transport in photosynthesis and respiration
	Ferredoxin	Electron transport in photosynthesis and N fixation
	Other Fe-S proteins	Electron transport in photosynthesis and respiration
	Nitrate and nitrite reductase	Conversion of nitrate to ammonia
	Chelatase	Porphyrin and phycobiliprotein synthesis
	Nitrogenase	N fixation
	Catalase	Conversion of hydrogen peroxide to water
	Peroxidase	Reduction of reactive oxygen species
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Zn	Carbonic anhydrase	Hydration and dehydration of carbon dioxide
	Alkaline phosphatase	Hydrolysis of phosphate esters
	RNA polymerase	Nucleic acid replication and transcription
	tRNA synthetase	Synthesis of tRNA
	Reverse transcriptase	Synthesis of single-stranded DNA from RNA
	Carboxypeptidase	Hydrolysis of peptide bonds
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Mn	O2-evolving enzyme	Oxidation of water during photosynthesis
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Arginase	Hydrolysis of arginine to ornithine and urea
	Phosphotransferases	Phosphorylation reactions
Ni	Urease	Hydrolysis of urea
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Cu	Plastocyanin	Photosynthesis electron transport
	Cytochrome oxidase	Mitochondrial electron transport
	Ascorbate oxidase	Ascorbic acid oxidation and reduction
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Multicopper ferroxidase	High-affinity transmembrane Fe transport
Co	Vitamin B12[a]	C and H transfer reactions
Cd	Carbonic anhydrase[b]	Hydration and dehydration of carbon dioxide
Mo	Nitrate reductase	Conversion of nitrate to ammonia
	Nitrogenase	N fixation

Adapted from Sunda (1989), with additional information from Raven et al. (1999), Frausto Da Silva and Williams (2001), and Wolfe-Simon et al. (2005). [a]: Cofactor in a number of enzymes. [b]: Has been found only in diatoms (Price and Morel 1990, Lane and Morel 2000).

2.3.3 Function of elements in consumers

In animal physiology, metal ions play a role in four major processes: enzymatic activation, homeostasis, signalling and communication and in enforcing physical structures such as mandibles (Mattson and Scriber 1987). In invertebrates, the haemolymph concentration of herbivores often reflects the ion concentration of their hosts, with high K and Mg concentration, whereas zoophages have high haemolymph or blood plasma Na concentrations (Mattson and Scriber 1987).

Joern et al. (2011) found that in Nebraska grassland habitats, besides N and P content, grass-feeding grasshopper densities were positively associated with Mg and Na content; forb- feeding grasshopper densities with Na and K and mixed- feeding grasshoppers with Mg, Na and Cu. Borer et al. (2019) found that under N fertilization, high Na plants were selectively consumed by invertebrate herbivores, thus suggesting a co-limiting role for Na in determining producers nutritional value. The elemental composition of pollen also varies considerably between species (Filipiak et al. 2017), and for several elements, the trophic stoichiometric ratio between producers and consumers has been found large enough to assume limitation for wild and honey bee populations (Filipiak et al. 2017, Filipiak 2018). For sodium (Na), the elemental mismatch was large enough to assume that this element is supplemented by intake of another nutrient source such as mud water, urine or tree sap. Metal deficiency in consumers can also be induced by plant secondary compounds. A high concentration of condensed tannins and phenolic compounds in the diet has been linked to Fe deficiency in the spruce budworm (Mattson and Scriber 1987). These studies indicate that for invertebrate herbivores, nutritional and ionomic mismatches beyond N and P can impact and shape population density and community structure. This in turn can shape community structure of higher order consumers through altered prey abundance mechanisms, without having direct ionomic consequences in these consumers (i.e. via food quantity instead of quality).

Vertebrate animals such as birds and mammals contain high fractions of Ca in their total body mass, as this this element is used for bone formation together with P. Vertebrates have much higher body Ca content than most invertebrates as this element is essential in bone formation through the precipitation of Ca-apatite minerals in the bone matrix. Herbivorous insects are therefore much less limited by Ca content of their diet, but much more by K content compared to vertebrates (Mattson and Scriber 1987). As an exception to this rule, invertebrates that use Ca as structural element in exoskeleton formation, such as isopods (and other crustaceans), millipedes and snails have high body Ca (Graveland 1995). These groups are good calcium sources for many vertebrates, but also susceptible to low pH and Ca deficiency. Calcium-rich invertebrates are hardly ever main food sources for vertebrates, but the Garden dormouse (*Eliomys quercinus*), feeding dominantly on millipedes, is an exception here (Kuipers et al. 2012).

Since many invertebrates have much lower Ca content than vertebrate predators, obtaining adequate amounts of Ca is especially challenging for insectivorous vertebrates. Ca availability is for these species often an important factor shaping animal populations and geographic dispersal (Sterner and Elser 2002). In birds, the overt symptoms of Ca deficiency such as poor egg shells and broken leg bones of chicks are clearly visible effects of Ca deficiency, but also included are decreased food intake, reduced activity and sensitivity, increased susceptibility to haemorrhages (in poultry: Leeson and Summers 2001), with undocumented effects on post fledging survival of wild birds. As most invertebrates have much lower Ca requirements, deficiency symptoms are much less likely to occur, although decreased availability may be reflected in lower tissue concentrations (Graveland 1995, van den Burg et al. 2014) Ca deficiencies in vertebrates are most prominent in studies on fish, amphibians and birds. The lack of studies on, for example, mammals cannot however be regarded as proof of absence of Ca deficiencies in mammals. Vulnerable species may for example be insectivorous bats, which only eat insects and no prey particularly rich in Ca (Barclay 1994, Adams et al. 2003, Booher 2008). In ungulate herbivores, however, deficiencies in Ca appears less important than for P, Na and sometimes trace elements, such as Cu and Se (Wallis de Vries 1998, Ohlson and Staaland 2001, Estevez et al. 2009, Becker et al. 2010, Pareja-Carrera et al. 2018, Borer et al. 2019, Huo et al. 2020). Deficiencies in herbivores are most likely to occur in regions characterized by nutrient poor, poorly buffered soils.

2.3.4 Summary conclusions

Metal ions and trace elements play an important role in enzyme functioning, cellular functioning and homeostasis and are thus essential for organismal functioning in both producers and consumers. Ionomic mismatch between producers and consumers seems to occurs frequently in the natural environment and the degree to which mismatches occur can be affected by nitrogen

deposition, either through elemental dilution resulting from increased growth under alleviation of N-limitation or resulting from acidification induced changes in metal uptake (i.e. reduced uptake of base cations, increased uptake of Al).

Nitrogen deposition can thus potentially either lower or increase elemental availability, thus resulting in changes in elemental availability and/or elemental ratios. The likelihood for these imbalances to occur strongly depends on geology and soil type. Studies providing evidence of elemental limitation in consumers are mostly confined to Ca deficiencies in avian predators, or P and/or trace elements in ungulates, for other groups of organisms the scarce studies are confined to correlative studies. This relative scarcity of studies focused on ionomic mismatch between producers and consumers cannot be regarded as evidence of absence of such relationships but instead stresses the need for further investigation.

2.4 Proteins and carbohydrates

2.4.1 Amino acids and proteins in producers

In plants and algae, nitrogen is assimilated into amino acids that are, in turn, the building blocks for proteins where most cellular nitrogen is allocated to (Geider and La Roche 2002, Sterner and Elser 2002, Fernandez and Galvan 2007, Masclaux-Daubresse et al. 2010, see also **Table 2**). First, nitrogen is assimilated into amino acids through the glutamine synthetase and glutamate synthase pathway, from which all other amino acids are synthesized. There are 20 amino acids used for synthesis of proteins, which differ in their nitrogen allocation, with arginine as being the relatively richest ($C_6H_{14}N_4O_2$) and tyrosine the relatively poorest ($C_9H_{11}NO_3$) in nitrogen relative to carbon (see also **Table 2**).

Some amino acids are only produced by plants, including leucine, isoleucine, methionine, phenylalanine, arginine, histidine, tryptophan, valine, threonine and lysine, and thus referred to essential amino acids (Kumar et al. 2017). Amino acids are used for the synthesis of a range of cellular compounds other than proteins, including nucleobases, chlorophyll, storage polymers, peptides and secondary metabolites.

2.4.2 Carbohydrates, fatty acids and lipids in producers

Plants and algae also synthesize a wide range of compounds lacking nitrogen in their molecular structure, such as carbohydrates, fatty acids and lipids (Sterner and Elser 2002). Synthesis of these compounds may indirectly depend on nitrogen availability. For instance, in a microalgal species, cellular carbohydrate, fatty acid and lipid content increased under nitrogen limitation due to a relatively excess of inorganic carbon and light (Gao et al. 2018).

2.4.3 Nutrient balancing in consumers: protein and carbohydrate intake regulation

Macronutrients include key biochemicals such as carbohydrates, lipids and proteins, and their composition may thus reflect the nutritional value of food for animals (Dadd 1960a, b, 1961, Behmer 2009). Cellular levels of these compounds in producers generally show distinct changes with the environmental availability of nutrients. For instance, limitation by macro-elements such as N and P may lead to the accumulation of carbohydrates and lipids, while limitation by N may reduce cellular levels of proteins (Gao et al. 2018). Heterotroph organisms are able to compensate macronutrient deficiencies by switching between complementary food items (Bernays and Bright 1991, Berner et al. 2005, Mayntz et al. 2005), or by increasing per capita food consumption in order to obtain sufficient amounts of nutrients (Simpson and Abisgold 1985, Raubenheimer and Simpson 1993, Berner et al. 2005, Hillebrand et al. 2009). For insects, the mechanisms of compensatory feeding for C and N (also indicated in paragraph 0) are based on carbohydrate and amino acid concentrations of the food measured by the insect during feeding, and have been investigated mechanistically using controlled feeding experiments within the so-called nutritional geometric framework (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993, Behmer 2009).

2.4.3.1 Nutritional geometry (NG)

Insects are known to be able to regulate the intake of C and N via intake regulation measured by carbohydrate and protein content of food items and internal carbohydrate and protein demand. The mechanism behind this intake regulation has been proposed by Raubenheimer and Simpson (1993), which propose that intake regulation is steered in part by haemolymph metabolites modulating the responsiveness of mouthpart receptors. Amino acids and sugars in food act as phagostimulants, which are detected by several types of receptors at tarsi and mouthparts. Haemolymph protein deficient locusts have been found to experience increased responsiveness of amino-acid sensitive receptors, and haemolymph carbohydrate deficiency induces increased responsiveness of sugar receptors (Abisgold and Simpson 1988, Simpson et al. 1991). These receptors produce signals to the central nervous system (CNS) either stimulating or inhibiting initiation of feeding.
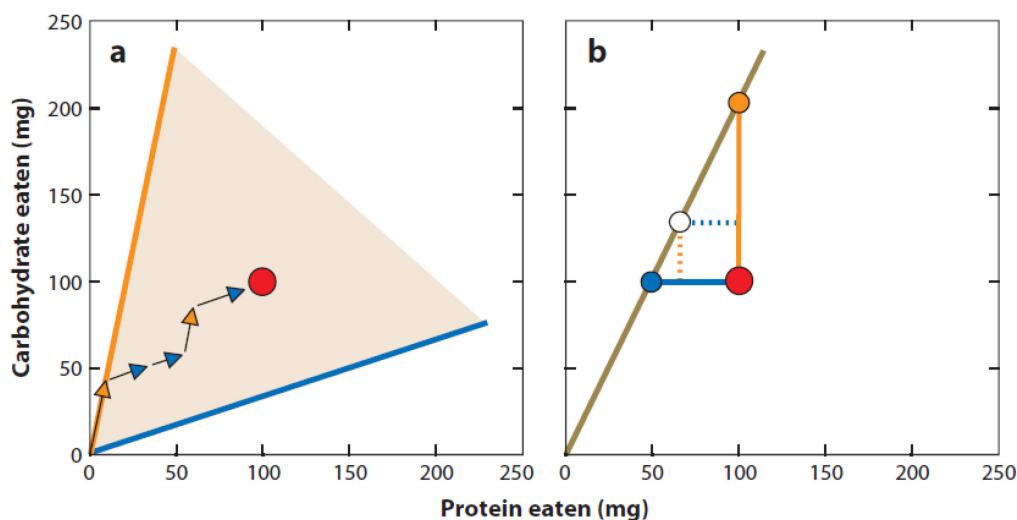
Figure 10. *Summary of the geometric framework (drawing from Behmer 2009). The intake target for protein and carbohydrate for an organism is defined as the amount and ratio of protein and carbohydrate where maximum fitness is realized, here depicted as a red dot in both graphs.*

Panel a: insects that are allowed to feed on two complementary nutritionally imbalanced food items are able to reach the protein:carbohydrate intake target by switching meals (arrows) from two food items differing in protein:carbohydrate ratio (nutritional rails; orange and blue line).
Panel b: when an insect is confronted with food of suboptimal quality without the ability to mix diets, the organism can choose to eat until it reaches the intake target for carbohydrate (blue dot), resulting in protein intake deficit, or to eat until intake target for protein (orange dot) is reached, resulting in excess carbohydrate intake, or to reach a point of compromise, relatively close to the intake target (white dot). The extent to which a consumer over- or undereats a nutrient represents a compromise which is often correlated with diet breadth.

Figuur 10. *Samenvatting van kernbegrippen van het 'geometric framework' (figuur uit Behmer 2009). Het 'intake target' voor eiwitten en koolhydraten voor een organisme wordt gedefinieerd als de hoeveelheid en verhouding van eiwitten en koolhydraten waar maximale fitness wordt gerealiseerd, hier weergegeven als een rode stip in beide grafieken.*

Figuur a: insecten die zich kunnen voeden met twee complementaire, maar in verhouding onevenwichtige voedselbronnen, kunnen het 'intake target' bereiken door (pijlen) te wisselen van beide voedselbronnen (de 'voedingsrails'; oranje en blauwe lijn). Figuur b: wanneer een insect wordt geconfronteerd met voedsel van suboptimale kwaliteit zonder mogelijkheid tot het mengen van voedselbronnen, kan het ervoor kiezen om te blijven consumeren totdat het de 'intake target' voor koolhydraten (blauwe stip) bereikt wordt, wat resulteert in een tekort aan eiwit, of om te eten tot het 'intake target' voor eiwit (oranje stip) wordt bereikt, resulterend in een overmatige inname van koolhydraten, of om voedselinname af te stemmen zodat een compromis tussen beide bereikt wordt, vaak zo dicht mogelijk bij het 'intake target' voor beide nutriënten (witte stip). De mate waarin een organisme één van beide nutriënten te veel of te weinig eet, is vaak gecorreleerd met de voedselstrategie (specialist vs. generalist).

This feeding mechanism acts both as a "brake-gas pedal" mechanism in controlling the total amount of ingested biomass under varying conditions of dietary nutrient content (compensatory feeding; Raubenheimer and Simpson 1993, Berner et al. 2005), and as a crude "tank-like steering"

mechanism in stimulating insects to preferentially feed on complementary food items (complementary feeding; Simpson and Raubenheimer 1993, Behmer 2009).

Under free choice conditions, herbivores are thus able to meet their intake requirement for both protein and carbohydrate by differential feeding on complementary food items (**Figure 10**a), whereas under suboptimal, no choice conditions, invertebrates are forced to over ingest one nutrient in order to meet the requirements for the other nutrient and vice versa, or to compromise by over ingesting to such extent that the costs of over-ingesting nutrient A equal those of under-ingesting nutrient B (**Figure 10**b).

2.4.3.2 Species-specific intake targets

Species can differ significantly in their optimal protein:carbohydrate intake target. Behmer and Joern (2008) showed that for seven coexisting grasshopper species, only two species showed overlap in their intake target, and concluded that all other species occupied a specific nutrient niche within the same habitat (**Figure 11**). Grasshoppers that were allowed to freely mix their diet managed to obtain optimal growth rate and development time as predicted by the intake target study (Behmer and Joern 2008). Species that have their nutrient targets in the extreme ends of the protein:carbohydrate ratio are expected to be more vulnerable to changes in plant protein or carbohydrate content, and are thus more vulnerable to changes in plant nutrient quality than species with their intake target near the centre of nutritional space. Specialist herbivores feeding on food relatively poor in carbohydrate or protein thus are especially vulnerable to changed macronutrient content of the host plant, especially when ratios change markedly. Grasshopper species tend to have their protein:carbohydrate intake at roughly equal amounts, while caterpillars tend to ingest protein to either equal or higher levels as carbohydrate (Behmer 2009), which might reflect different energetic demands for locomotion.
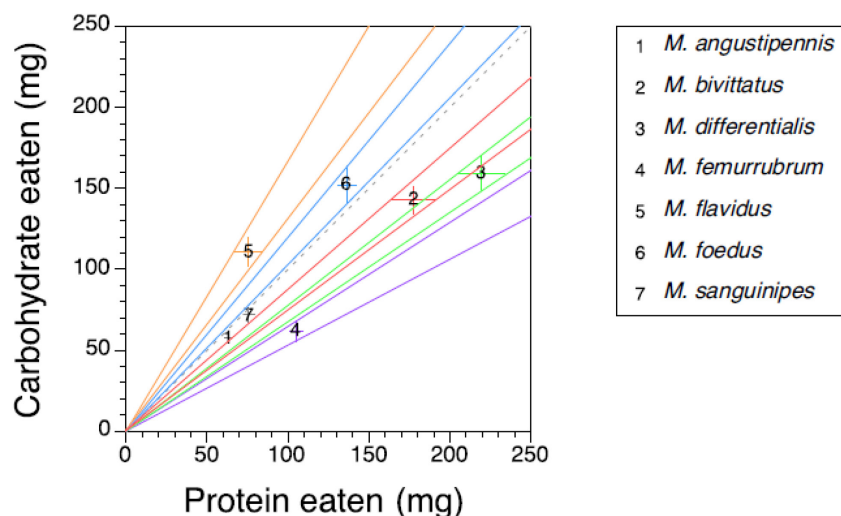
Figure 11. Protein–carbohydrate intake targets (means ± S.E.) for seven coexisting Melanoplus grasshopper species (figure from Behmer and Joern 2008). Species that are statistically different from one another have different colored error bars. The different colored solid lines define the range of nutrient space occupied by a particular species (as determined by the SEM of the intake targets). Overlap in nutrient space is only obvious for species 1 and 7.

Figuur 11. Intake targets (gemiddelde ± S.E.) voor zeven naast elkaar voorkomende Melanoplus-sprinkhaansoorten (figuur uit Behmer en Joern 2008). Soorten die statistisch van elkaar verschillen hebben verschillende gekleurde foutbalken. De verschillende gekleurde ononderbroken lijnen definiëren het bereik van de nutriëntruimte dat wordt ingenomen door een bepaalde soort (bepaald door de S.E. van de innamedoelen). Overlap in nutriëntruimte is alleen duidelijk voor soort 1 en 7.

2.4.3.3 Nutritional intake strategies

Expanding on the NG framework, experiments have been performed with several prepared food types differing in carbohydrate and protein level and protein:carbohydrate ratio (Raubenheimer

and Simpson 1993, Simpson and Raubenheimer 1993). Each food item or group of food items with a unique ratio (but differing in levels) represents a 'rail' in the nutrient intake plots (**Figure 12**).

The degree to which animals regulate their protein and carbohydrate intake can be visualized using these plots. The cumulative amount of nutrient A and B eaten by an animal, that is forced to eat a specific nutrient ratio and amount (i.e. 2:5 nutrient A:B food) is presented as a cross on the appropriate 'rail' (in this example the 2:5 rail). The square in each of the plots indicates the nutrient intake 'target', which is defined as the optimal cumulative amount of both nutrients eaten at which maximum growth is achieved. Note that in these experiments or plots, only compensatory feeding (i.e. increasing the amount of food eaten under conditions of low nutrient concentrations) is possible for the organism investigated, but complementary feeding is not (i.e. no animal is allowed to feed on two complementary food items).
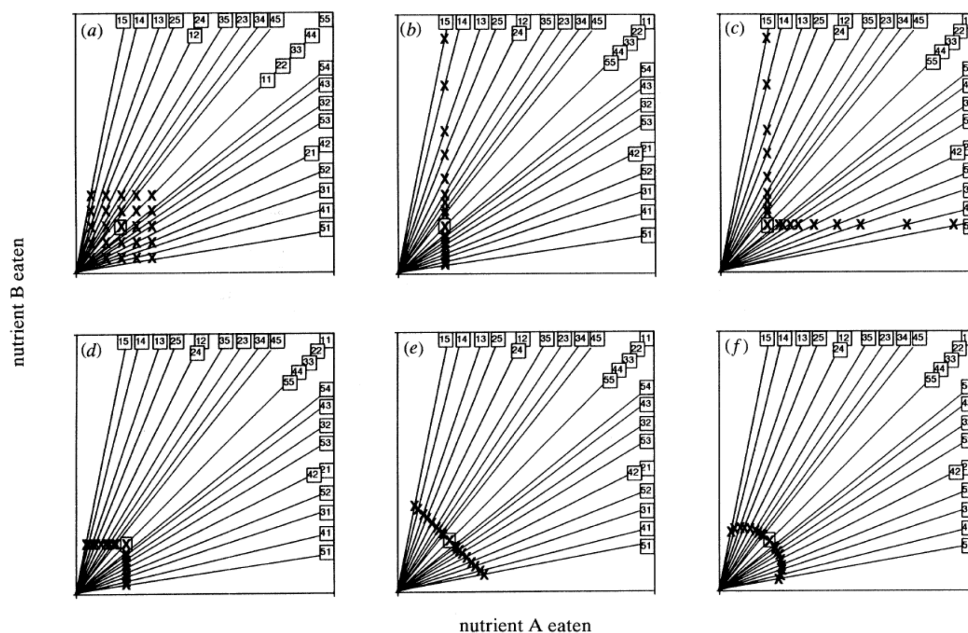
Figure 12. Nutrient intake plots in which animals are given one of 25 food types, containing each of 5 levels of nutrient A and B eaten, depicted by number combinations (i.e. 15 refers to food containing 1 unit of nutrient A and 5 units of nutrient B) (drawing from Simpson and Raubenheimer 1993). For explanation of the figures a-f, see main text.

Figuur 12. Nutriëntinname plots waarin dieren één van de 25 voedselsoorten krijgen aangeboden, die elk één van 5 gehalten aan voedingsstof A en B bevatten, weergegeven door cijfercombinaties in de figuren (bijv: 15 verwijst naar voedsel dat 1 eenheid nutriënt A en 5 eenheden nutriënt B bevat) (Figuur uit Simpson en Raubenheimer 1993). Voor uitleg van de figuren a-f, zie hoofdtekst.

Several outcomes are possible, indicating different regulatory mechanisms:

(1) **Figure 12**a: outcome if animals do not regulate intake for nutrient A nor B (no compensatory feeding occurs for nutrient A or B). Hence, cumulative intake (crosses) do not overlap in any of the rails.
(2) **Figure 12**b: outcome if animals strictly regulate nutrient A intake, but not nutrient B.
(3) **Figure 12**c: outcome if animals eat until they meet intake target for A and B, no costs are associated with ingesting excess A or B.
(4) **Figure 12**d: outcome if animals strictly regulate over intake of nutrient A or B, suggesting high costs of overeating A or B.
(5) **Figure 12**e: outcome if animals eat the sum of A and B to approximate the intake target, i.e. allowing for substantial overeating of A or B.
(6) **Figure 12**f: outcome if animals compromise between reduced intake and increased costs of ingesting excess nutrients. Animals eat until the point on the rail is geometrically closest to the intake target ("closest distance approximation"), minimizing costs in under- and overeating nutrient A and B.

The location of the cumulative nutrients eaten by each animal (the crosses in **Figure 12**) relative to the intake target (the square in **Figure 12**) in these plots thus describes the degree and strategy of nutrient intake regulation by a given animal, and provides a measure of the intake target of an animal for nutrient A and B.

These theoretical rules have later been confirmed using experimental data, resulting in three often encountered intake optimization rules (Behmer 2009):

- NI No Interaction rule (NI rule). Two nutrients do not interact, resulting in a straight line (**Figure 12**, panel b,c,d)
- Closest Distance rule (CD rule). The standard rule first described by (Raubenheimer and Simpson 1993) (**Figure 12**, panel f)
- Fixed proportion rule (FP rule). Assumes interaction between fitness costs associated between both food types: eating some excess of nutrient A is helpful in achieving maximum assimilation efficiency of nutrient B and vice versa (**Figure 12** panel e).

Subsequent studies have found that in most cases, consumers display either a CD or FP strategy, depending on the fitness costs associated with excess nutrient intake and whether fitness costs interact (Simpson et al. 2004). However, under specific circumstances, no regulation for macronutrients has also been found. Despland and Noseworthy (2006) found that the larvae of the forest tent caterpillar *Malacosoma disstria* do not regulate or compensate protein intake. This species synchronizes the larval stage with the growth flush of their host plants. In this specific period, nitrogen levels in leaves are predictably high, which might have resulted in no need for protein intake regulation (Behmer 2009).
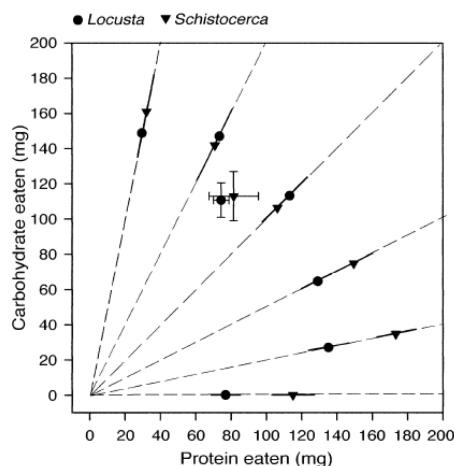
Figure 13. Intake arrays for the fifth nymphal stage of Locusta migratoria and Schistocerca gregaria *(from Raubenheimer and Simpson 2004), showing closest distance approximation (rule 6) as feeding strategy. Dashed lines represent nutrient intake rails for protein and carbohydrate with realized mean (± 1 SE) food intake for both species depicted on the rails. The two points with x and y directed (± 1 SE) error bars represent the intake targets for both species when nymphs were allowed for complementary feeding (i.e. feeding on two complementary foods).*

Figuur 13. 'Intake-arrays' voor het vijfde nymfenstadium van Locusta migratoria en Schistocerca gregaria (uit Raubenheimer en Simpson 2004), als voorbeeld voor een "closest distance approximation' (regel 6) als voedingsstrategie. Gestreepte lijnen vertegenwoordigen Nutriëntopname-rails voor proteïne en koolhydraten met de gerealiseerde gemiddelde (± 1 SE) opname voor beide soorten geplot op deze rails. De twee punten met x- en y-gerichte (± 1 SE) - foutbalken vertegenwoordigen de 'intake targets' voor beide soorten wanneer nimfen de mogelijkheid werd geboden om 'complementary feeding' toe te passen (d.w.z. voedsel aangeboden kreeg in de vorm van twee complementaire voedseltypen).*

Fitness costs depend on physiological constraints, such as the energetic costs of dealing with excess nutrients, but also on life history and behavioral adaptations. For instance, early instar nymphal *Locusta migratoria* and *Schistocerca gregaria* follow the CD rule (Raubenheimer and Simpson 1993, 2004; **Figure 13**), while at the adult stage, an intake array indicating FP rule emerges (Raubenheimer and Simpson 1993). In *Schistocerca*, solitarious phases follow the CD

rule, while gregarious individuals ingested more of an excess nutrient, resulting in a FP intake array (Simpson et al. 2002). This shift has been attributed to differences in the chance to encounter complementary food items in the environment, with solitary individuals having less chance to encounter complementary food items than gregarious individuals. This difference results in an evolutionary pressure stimulating the gregarious phase to ingest more excess nutrients. Therefore, generalist herbivores often show a FP intake strategy, while specialist herbivores should always strictly regulate intake in order to minimize associated fitness costs and hence should follow the CD strategy (Behmer 2009).

This outcome of different nutritional strategies between specialist and generalist (mobile) herbivores has also been hypothesized in a similar way in ecological stoichiometry theory (as discussed in par. 2.2.2.6), and shows similarities with the major axis in the adaptive trait continuum from Carnicer et al. (2012), (2013). These differences in nutrient acquisition strategies potentially have significant implications for specialist species encountering changed nutritional status of food or host plants, thus strengthening the hypothesis formulated in par. 2.2.3 that (low dispersing) specialist herbivores are more vulnerable to altered plant nutrient quality compared to (high dispersing) generalist herbivores.

2.4.4 Summary conclusions

Proteins, carbohydrates, fatty acids and lipids can be considered as the real currencies of the elemental nutrients N (proteins) and C (carbohydrates, fatty acids and lipids) that consumers need for organismal functioning. For both elements, N and C containing metabolites made by producers (amino acids, fatty acids), are essential for the consumers (they cannot metabolize all of these molecules themselves). For consumers, the carbohydrate:protein ratio is thus a better measure of macronutrient quality than the C:N ratio of the producer (which can however be used as a proxy). As for C and N, the ratio in which carbohydrates and proteins exist in food items are thus determining the nutritional value for the food items. And as for C:N:P stoichiometry, these ratios are not linearly linked to either higher carbohydrate of protein content, but instead have an optimum ratio at which maximum growth can be achieved.

Experiments in grasshoppers made insightful that different species have distinctly different intake targets, both in absolute amounts and in protein:carbohydrate ratios. This means that when protein:carbohydrate ratios change as a result of increased N availability, some species will benefit (i.e. producer tissue protein:carbohydrate ratio moves towards their intake target) while other species will suffer (i.e. producer tissue protein:carbohydrate ratio moves away from the intake target). Under chronically increased N-deposition above critical levels, this may thus result in a general decrease in consumer biodiversity, as under such conditions a reduced variation in the producer protein:carbohydrate ratio in the ecosystem is likely to take place.

Consumers do not passively ingest food items and deal with the nutritional protein:carbohydrate ratio that is offered in these food items; they can actively monitor their own amino acid and carbohydrate stores and measure the amino acid and carbohydrate contents of the food that they are ingesting. Using this information, consumers are thus capable in adapting their foraging behavior when offered food of general low nutritional value by increasing their ingestion rate (compensatory feeding) and when offered food of suboptimal nutrient ratios by selectively feeding on complementary food items, when available (complementary feeding). Furthermore, experiments have made it clear that consumers actively balance their food intake rate via different intake strategies. Nymphal stages, and low mobile specialist consumers in particular, balance their intake to such a degree that it matches the intake target as close as possible (closest distance optimizers). Adult life stages and highly mobile generalist species in particular often over-ingest a given nutrient to some degree (fixed proportion optimizers), as this may be beneficial for locomotion (i.e. eating excess carbohydrate) or may be counterbalanced by finding a complementary food item in the near future.

The nutrient balancing strategy used by a consumer thus determines the sensitivity to N deposition mediated changes in protein:carbohydrate ratios in producers. In parallel to the hypotheses posed

in the C:N:P stoichiometry section (par. 2.2, in particular 2.2.3) these changes would affect (low-dispersing) host-plant specialist to a much greater degree than (high-dispersing) host-plant generalists. Moreover, the most vulnerable life stage to such changes is hypothesized to be the juvenile or larval stage, since in these stages nutritional demands are highest while dispersal capabilities are often lower than in the adult life stage.

2.5 Micronutrients: essential metabolites

2.5.1 The organismal significance of micronutrients

Micronutrients are essential components of animal food, and can refer to trace elements (in this report, trace elements are covered in a separate section; see paragraph 2.3), essential fatty acids, vitamins, and amino acids. 'Essential' implies that consumers need these elements (often in low amounts) for their body functioning or, in case of complex compounds such as amino acids, vitamins, etc., that consumers cannot synthesize these themselves. Trace elements are always essential (i.e. must be in adequate amounts obtained by food intake). However, due to physiological differences or differing bacterial symbiotic relationships, different species do have different requirements when it comes to essential metabolites. Biological compounds (e.g. vitamins) may be synthesized by the animal, or produced by gut flora (e.g. Chapman 1998), in which case, it is not essential for these compounds to be sufficiently supplied by food items. Some animals still need particular precursors and synthesize other compounds (vitamins, amino acids) therefrom, but this may not be true for other species.

2.5.2 Micronutrient deficiencies: an understudied topic in ecology

An initial literature survey was performed to discover the major micronutrient issues in relationship with nitrogen deposition (see Appendix 1). Overall, the number of relevant papers on different micronutrients is very low, often zero or close to zero. Removing the term "ecosystem" from the search strings increased the number of hits substantially, but resulted mostly in a high number of papers related to humans or animal husbandry studies. Indeed, by far most studies on micronutrient limitations in animals have been conducted in the context of livestock and husbandry (e.g. McDonald et al. 1995, Leeson and Summers 2001). This body of literature comprises studies into the functions of micronutrients as well as diagnostic research into the causes of lowered viability and/or reproduction. In an ecological context the latter are most relevant, as these papers indicate that even under a controlled nutrient supply regime, animals can incur adverse effects of micronutrient deficiencies. This may indicate that in an (uncontrolled) natural environment, micronutrient deficiencies are likely to occur, although reports are relatively scarce. In humans, micronutrient deficiencies have been researched more profoundly, and this has led to the conclusion that 'inadequacy of diets leading to deficiency of various micronutrients is very widespread, although signs or symptoms are often lacking'. Micronutrient deficiency is therefore also referred to as the "hidden hunger" (Dijkhuizen and Wieringa 2001, based on WHO/FAO reports).

If signs or symptoms are often lacking or subtle, it is not obvious that such deficiencies would be widely studied in animals in the wild. Also, the outcome of animal husbandry studies may be relevant in an economic context (profit maximizing is the focus), but not in an ecological context (main focus is on species persistence or ecological stability), which limits the practical use of many of these studies for ecological interpretation. Failed egg diagnostics in bird species often shows embryonic anomalies that, based on knowledge obtained from poultry studies, have most likely arisen from micronutrient deficiencies (Van den Burg 2017a). Next to the often subtle effects of deficiencies, the uncertainties in this observation delineate a second cause of underrepresentation of ecological micronutrient studies: it is often time and money consuming as well as scientifically challenging to prove effects of shortages of micronutrients.

The hierarchic level in a food chain that is occupied by a model organism is also relevant to the likelihood and nature of micronutrient deficiencies (e.g. van den Burg et al. 2014). Within food chains, micronutrients may accumulate or get lost at higher trophic levels. For example, riboflavin

(B vitamin) accumulates from plants to insect herbivores, and further towards the eggs of avian insectivores, but then gets lost higher up the food chain (van den Burg et al. 2014). So, the choice of model organisms is crucial in micronutrient studies.

Not only the hierarchical position in the food chain matters, but also the exact food choice and physiological capabilities determine the outcome of feeding on food items with a reduced quality in terms of micronutrients. For example, a particular micronutrient may be an essential component of the diet in one species of insect, but not in another (e.g. Chapman 1998). Likewise, apparent details in (lack of) dietary supplementation may result in severe problems for one species but not for another: calcium shortage in acidified forests in songbirds may be lifted by consuming woodlice, but for example Great tits (*Parus major*), unlike many other bird species, simply do not eat woodlice (Graveland and Vangijzen 1994). Such characteristics of animals represent a fourth challenge in hypothesizing and testing micronutrient- based mechanisms in nutritional (population) ecology. The key to unravel this complexity is to have sufficient knowledge on the general properties and physiology of the micronutrient of choice in producers and consumers, and of the specific adaptations employed by the specific model organism under investigation (see also section 2.7). Important aspects on a species-level are whether the component is essential in the diet, what the amount of this component is in total food intake, if it can be stored and re-allocated upon increased demand within the animal body (or not).

Given the complexities above, the apparent lack of studies showing effects of micronutrients in ecology, does not indicate that these do not exist. Where species are on the brink of population stability or decline, even micronutrient deficiencies without obvious dramatic effects may contribute to tip the balance towards an unfavorable direction (in accordance with the above-introduced 'hidden hunger' concept in humans). In general, we lack studies on the effects on animal performance in the wild of minor-impact deficiencies (and the same is true for minor-impact toxicities). In conclusion, we expect the effects of micronutrient limitations to be underestimated in current literature in relationship with their true occurrence, irrespective of how important these limitations may be for the stability of animal population sizes.

2.5.3 Micronutrients in producers

2.5.3.1 Amino acids

Amino acids are the building blocks of protein (par. 2.4). Protein is not a micronutrient, but individual amino acids are considered micronutrients, especially if they are essential components of the diet. A relative shortage of a single essential amino acid can limit the production of particular proteins and thereby disrupt the cellular processes these proteins are governing (e.g. enzymes).

There are many studies linking nitrogen deposition to changes in amino acid physiology in plants. The composition of free amino acids changes markedly as a result of nitrogen deposition (Perez-Soba et al. 1994, Huhn and Schulz 1996), and as such the accumulation of free amino acids, especially arginine, is used as a bio-marker for nitrogen deposition (e.g. Pitcairn et al. 2003). However, the total amino acid composition, after protein breakdown, does not seem very different between plants under high or low nitrogen deposition (Nijssen and Siepel 2010, van den Burg et al. 2014). As most amino acids are components of protein and Rubisco is the most common protein in plants which is also used as amino acid storage protein, this may not be surprising. However, reductions of rare amino acids which do not stand out in the entire make-up of plant protein may be critically important for herbivores that rely on such amino acids to be available in their food at adequate amounts (i.e. a reduction of a rare amino acid by 50% may only represent a 1% change in the total amino acid make up of plant protein).

2.5.3.2 Fatty acids

Shifts in the composition of the primary producer community, for example due to eutrophication and nitrogen deposition, drive the variation in fatty acid availability in aquatic ecosystems. Fatty acid deficiencies are mostly reported in aquatic ecosystems and involve linoleic acid, linolenic acid, or long-chained poly unsaturated fatty acids (PUFA's, poly unsaturated fatty acids, with 2 unsaturated carbon bonds, and HUFA's, highly unsaturated fatty acids, with 3 to 6 unsaturated

carbon bonds) (Gomes et al. 2016, Twining et al. 2016). Of the HUFA's present in aquatic food chains, docosahexaenoic acid (DHA) is the dominant type in animals.

Terrestrial plants contain PUFA's but little HUFA's, presumably because the double bonds in these compounds are easily broken up by oxygen or oxygenated radicals. In water, in which oxygen diffusion is strongly reduced, primary producers are typically rich in HUFA's and DHA (Twining et al. 2016). This is, however, strongly dependent on the diverse phylogenetic relationships of primary producers: diatoms and dinoflagellates for example contain low levels, whereas chlorophytes and cyanobacteria are rich in these compounds (Gomes et al. 2016).

2.5.3.3 Vitamins

There are very few records of problems concerning vitamin deficiencies that can somehow be related to nitrogen input in ecosystems. In some cases, like vitamin A, B2 or B12 deficiencies in animals, the underlying causes can be related to soil acidification and hence low mineral nutrient availability for plants, or physiological problems in producers arising from amino acid assimilation limitations. So, in these cases, the problems can be secondary effects of nitrogen deposition.

An exception is thiamine (vitamin B1), which deficiency has been identified as a problem especially in aquatic ecosystems. The key causal factor of thiamine deficiencies is not its production, but that of thiaminases (i.e. enzymes that break down the vitamin) being used abundantly as anti-feedants in aquatic ecosystems, but only rarely in terrestrial plants (Kraft and Angert 2017, Harder et al. 2018). Thiamine is a co-factor in many physiological pathways, such as carbohydrate and amino acid metabolism (Ejsmond et al. 2019). Its key functions make thiamine a vital compound for the proper functioning of plants, many microbes, and animals. Thiamine production is limited to plants, fungi and microbes, although many bacteria do not have this capability (Ejsmond et al. 2019). The pathways of thiamine production differ among taxa: some taxa are able to completely synthesize thiamine, others need precursor molecules (Kraft and Angert 2017). Thiamine is stable in acidic environments and can also be found adhering to negatively charged soil particles in terrestrial and aquatic ecosystems. Due to molecular degradation of thiamine, its precursor molecules are also present in the environment. For animals however, the complete vitamin is an essential micronutrient (Kraft and Angert 2017).

2.5.4 Micronutrients in consumers: organismal function and deficiency symptoms

2.5.4.1 Amino acids

Amino acids make up protein. A general shortage of protein in the food of animals can thus also be regarded as an amino acid deficiency. In this review however, such shortages are discussed as a macronutrient (overall nitrogen) problem, and not a micronutrient deficiency (see paragraphs 2.4 and 5.4). Here, amino acid deficiencies in terms of food quality are restricted to problems regarding the relative amino acid composition of producers. As such, shortages of particular amino acids are not integrated within the nutritional geometry framework, as this framework seeks to balance carbohydrate and amino acid intake, but does not govern a balanced consumption of all types of amino acids. This is also hardly possible, as the amino acids are often not present in the food as such, but as proteins the composition of which cannot be assessed by the herbivore before digestion.

Many studies on the effects of imbalanced amino acid nutrition have been conducted in livestock settings. Amino acid deficiencies in poultry describe a wide array of embryonic anomalies in avian eggs (Romanoff and Romanoff 1972), the diversity of which is expected to reflect the varying nature of amino acid shortages: a deficiency may be caused by a single essential amino acid or several amino-acids simultaneously. Homologous to elemental stoichiometry and nutritional geometry, also excesses of particular amino acids relative to demand give an unbalanced mixture of amino acids, which may lead to embryo failure (Romanoff and Romanoff 1972).

In nature, symptoms of amino acid deficiencies in avian eggs are not rare, but to attribute these to nitrogen deposition requires thorough understanding of the reproduction strategy, dietary and other sources of amino acids, and how these are affected by nitrogen deposition (Van den Burg in

press). Because such data are hardy available it is still impossible to estimate the ubiquity of amino acid deficiencies in wild birds due to nitrogen deposition. At the Veluwe inadequacies food quality have been observed at each trophic level of the forest food chain from producers to top predators, and amino acid limitations were found in producers, insectivores and predators (van den Burg et al. 2014).

For aquatic systems, Taipale et al. (2018) found that although levels of essential amino acids differ among the food types available to zooplankton, there is no effect of food type on essential amino acid levels in *Daphnia.* The authors suggest that this lack of effect may be caused by the efficiency of essential amino acid accumulation in *Daphnia*, but do not consider the possible role of specific amino acid synthesizing bacteria (see par. 2.7). However, population development and fecundity of *Daphnia* have been shown to be reduced 10-fold when *Daphnia* is stripped from its bacterial symbionts (Peerakietkhajorn et al. 2015). So, an alternative hypothesis is that zooplankton is not susceptible to essential amino acid limitations due to bacterial symbiosis. If this is a common trait in zooplankton, amino acid deficiency may not occur in the aquatic food web, as a result of the key position of zooplankton and their bacteria in aquatic food chains.

2.5.4.2 Fatty acids

HUFA's (for explanation, see paragraph 2.5.3.2) are fatty acids necessary to maintain membrane fluidity, but are also precursors to vital signalling molecules such as the eicosanoids, which have key functions in the regulation of inflammatory diseases (Gomes et al. 2016). In humans, shortages of HUFA's result amongst others in decreased growth, weight loss, impaired sight, and growth of fatty liver tumors (Twining et al. 2016). In zooplankton, growth rates and fecundity are reduced by HUFA deficiency. Fish larvae are also particularly susceptible and show reduced growth and survival (Taipale et al. 2018). As HUFA's are vital to the proper functioning of the animal body, they are bio-accumulated and preferentially stored over other fatty acids (Twining et al. 2016). Also, in a situation in which fats are burned for energy, HUFA's are spared. Therefore, higher order carnivores are expected to be much less prone to fatty acid deficiencies compared to first order consumers (Larson et al. 2017).

Animals that have diets typically rich in HUFA's and/or DHA have poor to no capacity to make these compounds from PUFA precursors (Twining et al. 2016). This includes aquatic first order consumers (Larson et al. 2017), but also obligate terrestrial carnivores such as cats (Twining et al. 2016). Similarly, some groups of parasitic invertebrates, such as helminths cannot synthesize fatty acids and thus fully rely on the fatty acid intake and metabolism of their hosts fatty acids (Mondal et al. 2016). Such groups of animals are susceptible to HUFA deficiencies if the HUFA content of their prey (or host) drops. This may be due to eutrophication which can cause blooms of diatoms, which contain little HUFA's (Gomes et al. 2016).

For many other animals, HUFA's are semi-essential as they can be produced from PUFA precursors, but the performance of these animals (including humans) is much better if there is a dietary source of HUFA's and/or DHA (Twining et al. 2016). Also in humans, dietary HUFA restriction causes deficiency symptoms, as the efficiency of HUFA production from PUFA's is too low to keep up with the demand. Due to the low production rate of HUFA's these types of fatty acids are considered essential nutrients, even though consumers are to some degree capable of synthesizing these nutrients. Virtually nothing is known about the efficiency of HUFA synthesis in wild animals.

2.5.4.3 Vitamins

2.5.4.3.1 Thiamine, vitamin B1
Thiamine deficiency can lead to impaired functioning of mitochondria, nervous system and the immune system, ultimately resulting in reproductive failure, increased mortality and possibly also population decline in case of systemic thiamine deficiency in consumer populations (Ejsmond et al. 2019). Thiamine deficiency causes high mortality among the early life stages of many fish species, causing their populations to decline (Harder et al. 2018). However, some fish species appear to accumulate thiaminases from their food without any detrimental fitness effects (Kraft and Angert

2017). Instead, they are able to accumulate these enzymes as antifeedants for higher order carnivores, resulting in increased mortality of fish eating birds and crocodiles. As such, thiaminase induced thiamine deficiencies can alter population dynamics throughout the entire food chain.

2.5.4.3.2   Riboflavin, vitamin B2

Riboflavin is a precursor molecule of two redox-coenzymes. Riboflavin is a precursor molecule of two redox-coenzymes. These are essential in the Krebs-cycle and also in the beta-oxidation of fatty acids (McDonald et al. 1995). Riboflavin concentrations in eggs of Great tits are lower in forests affected by N deposition and soil acidification, and embryos in failed eggs of Sparrowhawks often show signs of vitamin B2 deficiency (van den Burg 2009). The underlying problem is not the production of the vitamin itself. The accumulation of the vitamin in firs order consumers is dependent on the availability of riboflavin binding proteins; shortages higher up the food chain may thus result from amino acid deficiencies (van den Burg et al. 2014). As such, riboflavin deficiency symptoms in (higher order) consumers are here regarded as a side-effect possibly caused by amino acid deficiencies (van den Burg et al. 2014).

2.5.4.3.3   Cobalamine, vitamin B12

Vitamin B12 (cobalamine) is an animal vitamin, and plant sources of this vitamin are exceptionally scarce (Milewski and Diamond 2000, Leeson and Summers 2001). Plants do not synthesize this vitamin, and the organic parts of cobalamine are produced by some bacteria (Leeson and Summers 2001). This implies that animals also derive this vitamin from symbiotic microorganisms or the animal matter that they feed on. Among the insects, some herbivores may not contain such symbionts, and indeed, some insects contain no detectable amounts of cobalamine (Wakayama et al. 1984). However, for many other insects, other invertebrates (Halarnkar et al. 1987) and all vertebrates, cobalamine is of vital importance for organismal functioning (Leeson and Summers 2001). Cobalamine functions in several enzyme systems involved in transferring or creating methyl groups. It is functionally associated with folic acid (another B vitamin), methionine and choline. As such, it is involved in DNA and amino acid synthesis, fatty acid and carbohydrate metabolism, red blood cell synthesis, and myelin production in the nervous system (Leeson and Summers 2001).

A known pathway leading to of cobalamine shortage is Co (cobalt) deficiency (Siepel et al. 2009), as this trace metal is a crucial component of the vitamin. Soil acidification may lead to leaching of Co and thus to reduced Co availability (Daroub and Snyder 2007), and ultimately to cobalamine deficiency. Evidently, without sufficient Co, bacteria cannot synthesize cobalamine, and hence Co deficiencies have been related to cobalamine deficiencies in grazing animals (McDonald et al. 1995), such as Mouflon *Ovis ammon* in National Park Hoge Veluwe (Siepel et al. 2009). As Co has been identified as driver of microbial fermentation of plant fibers in grazer gut systems (Milewski and Diamond 2000), grazers may be particularly susceptible to low Co availability. This is still rare as cobalamine is actively stored in liver tissue (McDonald et al. 1995), which is truly exceptional for a water soluble B-vitamin. Stored cobalamine may be able to sufficiently supply the vitamin for months to several years in the individual. Cobalamine deficiency can thus be regarded as a possible secondary effect of nitrogen deposition, promoted by increased soil acidification.

2.5.4.3.4   Vitamin A

Apart from its well-known function in vision, vitamin A is also important in growth and reproduction, maintenance of mucous membranes and in controlling cerebrospinal fluid pressure (Leeson and Summers 2001). Vitamin A deficiencies are not likely to arise from a lack of dietary intake of vitamin and/or its precursors in wild animals, but could be a result of Zn deficiency (Smith et al. 1973, Smith 1980, Dijkhuizen and Wieringa 2001). Zn is required to synthesize active vitamin from its precursor. As such, soil acidification and leaching can potentially result in low Zn availability in plants. However, this is not commonly observed as plants actively increase their zinc uptake efficiency at low soil concentrations (Humayan Kabir et al. 2014), and the solubility of Zn increases under increased soil acidity. Vitamin A and/or Zn deficiencies in wild fauna may sometimes occur, for example among failed avian eggs, but seems thus far of low ecological significance.

2.5.5 Summary conclusions

Although micronutrients are not well-studied in wild animals from the perspective of N deposition, reviewing livestock, veterinary and human studies warrants the conclusion that several pathways for micronutrient limitations may exist. Another conclusion that can be drawn from these studies is that of the suite of micronutrients limitations which cause harm to animals, only a few can be related to N deposition in theory or in practice.

N deposition results in acidification, eutrophication, and in particular N-enrichment of plants. All of these primary effects can have significant consequences for food quality for animals in terms of micronutrients. Especially in aquatic communities, problems in terms of micronutrients are caused by eutrophication and shifts in the composition of algal primary producers therefrom (fatty acid deficiency, thiamin deficiency). In terrestrial ecosystems, micronutrient limitations typically arise because the quality of plants themselves change. Changes in plant quality may be caused by N-enrichment and relate directly to micronutrient levels (imbalance in amino acids) or, due to acidification, relate to insufficient elemental uptake that are essential in micronutrient synthesis (i.e. Zn for vitamin A, Co for vitamin B12).

Animals prone to risk micronutrient deficiencies are top-predators (in case of amino acids, riboflavin, thiamin) or first order consumers (in case of fatty acids, vitamin A and vitamin B12). Compounds that do not accumulate well in food chains (or even destroyed in the reported case of thiamin deficiency) at lower food chain levels pose risk factors to predators. The animal micronutrients (vitamin A, vitamin B12) as well as essential fatty acids are typically in sufficient supply to top predators, and their deficiencies especially occur if food quality is insufficient from producers to first order consumers.

A further conclusion is that N deposition driven micronutrient deficiencies will often impact populations of specific species on a regional scale. Regions which are especially vulnerable are those with an exceptionally high (accumulative) N deposition and in regions consisting of ecosystems naturally poor in plant nutrients. Of the micronutrients reviewed here, there are already some examples flagging out on particular species in particular regions.

2.6 Antifeedants and phytotoxins

2.6.1 Allelochemicals: producers defence to consumer's performance

As illustrated in the previous sections, nutritional quality is determined by the elemental, macro and micronutrient supplies by the producers and the demands of these by the consumers. Yet, other biochemical compounds produced by autotrophs (allelochemicals) may have negative consequences for consumers, and thus play a completely different role in determining the overall nutritional quality of producer tissue. Allelochemicals involved in producer defense against consumers can be subdivided in two groups, irrespective of their chemical composition (van Genderen et al. 1996). Allelochemicals that (mainly) function via impairment of nutrient uptake efficiency are here referred to as antifeedants, while those that function via impairment of vital organismal functioning of consumers (eg. blocking or disruption signal transduction in synaptic transfer) are referred to as phytotoxins.

In general, N containing allelochemicals act more often as phytotoxins, leading to impaired functioning of consumers through direct toxic effects on organismal functioning (eg. cyanide), whereas strictly carbon based allelochemicals can either act as antifeedants (eg. (condensed) tannins, latex forming polyisoprenes, even lignin can be regarded as an effective antifeedant), or as phytotoxins (e.g. flavonoids, terpenes, steroids). In aquatic systems, phytoplankton can also produce a range of toxic compounds that may, in some cases, negatively affect grazers, though the ecological role of these compounds as grazing deterrents is still under debate (Cusick and Sayler 2013, Ger et al. 2014). Common toxins include N-rich alkaloids such as saxitoxin, produced by cyanobacteria and dinoflagelaltes, as well as peptide based microcystins and nodularins, produced by cyanobacteria (Gobler et al. 2016).

Nitrogen containing allelochemicals are mainly synthesized via amino acid synthesizing pathways and include non-proteinogenic amino-acids, cyanogenic glycosides, alkaloids and glucosinolates (Palo and Robbins 1991, van Genderen et al. 1996). Their concentrations are usually low relative to bulk nitrogen, but they can be highly toxic. Another group of N containing allelochemicals are amino acids not used in protein synthesis. These non proteonomic amino acids are common and many types exist (250 have been identified in plants). They are mere metabolites or function as storage or transport amino acids, or are important in signaling and stress responses (Vranova et al. 2011). The non-proteinomic amino acid ornithine for example is crucial in plants in the biosynthesis of arginine (Winter et al. 2015). Blooms of cyanobacteria are also known to produce an array of N containing toxins, including harmful non-proteinomic amino acids (Cox et al. 2018, Rodgers et al. 2018, Dunlop and Guillemin 2019).

As indicated above, nitrogen can be a limiting nutrient in natural aquatic and terrestrial systems. Consequently, there is a trade-off between the synthesis of nitrogen containing allelochemicals and amino acids used in proteins for growth (Palo and Robbins 1991). Not surprisingly, plant species adapted to N rich environments, as well as plant species with N_2 fixing symbionts (mainly leguminous species) often contain these N-containing allelochemicals, whereas plant species adapted to N poor environments usually lack these N-containing allelochemical synthesizing pathways (van Genderen et al. 1996). Nitrogen deposition has indeed been found to increase plant N that is not incorporated in plant protein, which is then assumed to be present in the form of N containing allelochemicals in plant tissue (van den Burg et al. 2014). Whether and to what degree this increase in non-protein nitrogen affects consumer performance, is however highly dependent on the type of N-containing allelochemical this excess N is stored in the plant.

Besides N-containing allelochemicals, plants can also produce a range of other C-based allelochemicals (e.g. tannins, phenolics, terpenes, flavonoids, cardelonide glycosides, stilbenes), which are often expected or found to have contrasting responses to the degree of nitrogen availability (Hofland-Zijlstra and Berendse 2009, Bandau et al. 2015, Felix et al. 2016, Campbell and Vallano 2018, Nybakken et al. 2018). Drought stress has often been found to interact with nitrogen supply in determining the levels of C-based allelochemicals, in which cases found to act as a prerequisite for lowered leaf allelochemical concentration under elevated N supply (Thomas and Schafellner 1999, Jamieson et al. 2013).

2.6.2   Consumers response to allelochemicals

2.6.2.1   *Allelochemical driven species diversification and host plant specialization*
During the course of evolution, many consumers co-evolved with host plant species (Farrell and Mitter 1990, Agrawal et al. 2012; **Figure 14**), and often have co-evolved physiological mechanisms that significantly reduce or nullify the phytotoxic effects of the allelochemicals produced by the host plant (Agrawal et al. 2012). This co-evolution may be an important driver in the diversification of consumer species and acts as a selective pressure towards host plant specialization. For specialist consumers, it is therefore less likely that increased concentrations of allelochemicals in their hosts will lead to highly impaired functioning, although they often show reduced fitness under increased allelochemical concentrations of their host plant (Agrawal et al. 2012). Sustaining physiological mechanisms to cope with toxic allelochemicals are costly and will reduce the growth potential of the species. Thus, N-deposition mediated reduced concentrations of allelochemicals will probably result in a relatively moderate increase in performance, while increased concentrations will result in a moderate decrease in performance for specialists. In contrast, changes in phytotoxin concentrations will often result in stronger consumer growth response for generalist consumers, since these organisms usually show very poor growth and survival on these plants (Agrawal et al. 2012), while at the same time they are not limited in growth potential by physiological adaptations to cope with these compounds.

This contrasting response of specialists versus generalists mainly applies to plants that utilize a strategy of producing phytotoxins. For plant utilizing an antifeedant strategy both specialists and

generalist species will show comparable growth responses to changed allelochemical concentrations in the host plant, but differences in morphology (i.e. gut volume and/or surface; microbial symbiosis) and physiology might still modulate their growth response to allelochemical concentrations.
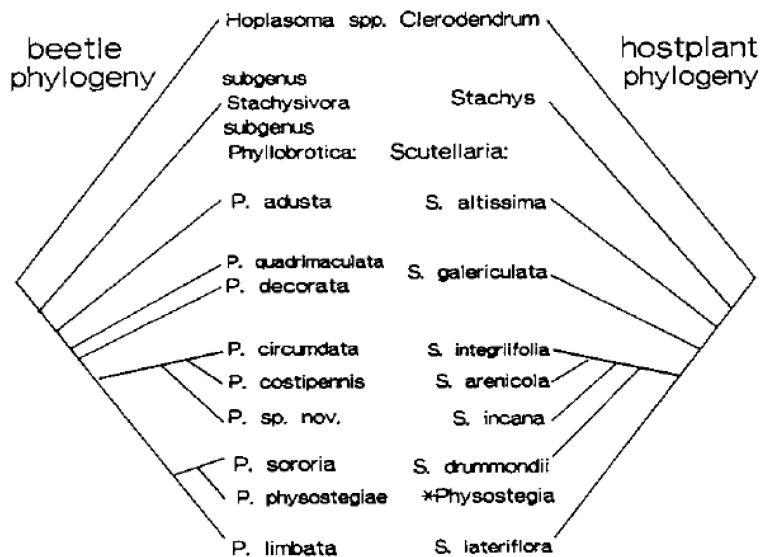
Figure 14. *Comparison of beetle and hostplant phylogenies published by Farrell and Mitter (1990). Phylogeny comparison shows almost complete similarity, except for* Physostegia *and its associated specialized beetle species.*

Figuur 14. *Vergelijking van de fylogenetische stambomen van kever en waardplant, gepubliceerd door Farrell en Mitter (1990). Er bestaat een bijna volledige gelijkenis, behalve voor Physostegia en de bijbehorende gespecialiseerde keversoorten.*

2.6.2.2 Effect on consumers performance

Since many studies on the effect of allelochemicals on consumer performance exist with low specific relevance to nitrogen deposition, this report did not attempt to provide a complete review on this subject. Here, we focus on a number of case studies illustrating different pathways and outcomes of possible nitrogen deposition effects on allelochemical – consumer interactions.

2.6.2.2.1 N containing allelochemicals

Campbell and Vallano (2018) report that under high foliar NO_x exposure, plant tissue alkaloid concentrations rise, resulting in reduced caterpillar performance. High root nitrate exposure did not have an effect on foliar alkaloid levels. Gleadow and Møller (2014) describe that particularly the combination of drought stress and high nitrogen availability strongly increases cyanogenic glycosides, a N-containing phytotoxin.

N deposition may also lead lead to increases in non-proteionomic amino acids in plants. Some of these may act as phytotoxin, such as L-Canavanine found in some legumes, which is erroneously incorporated in proteins, rendering them dysfunctional (Staszek et al. 2017). L-Canavanine is mainly found in seeds and young sprouts, which further suggests their role as phytotoxin. Beta-alanine in nectar of *Gentiana lutea* has been observed to reduce the performance of bees (David et al. 2019). Several papers describe deleterious effects of non-proteionomic amino acids which intensify up into the food chain due to bioaccumulation (Dunlop and Guillemin 2019). However, in eggs of Sparrowhawks on the Veluwe, no detectable amounts of non-proteionomic amino acids were found (pers. Obs. A. van den Burg).Harmful non-protein amino acids from blooms of cyanobacteria cause neurodegenerative health effects, also in humans (Cox et al. 2018, Rodgers et al. 2018, Dunlop and Guillemin 2019; Figure 15). The occurrence of such algal blooms is associated with eutrophication (N and P) and water temperature (Jankowiak et al. 2019).

2.6.2.2.2 C based allelochemicals

Increasing tannic acid (an antifeedant concentration in a nutritional geometry study with *Locusta migratoria* only affected locusts feeding on imbalanced foods, and had no influence on intake or performance when nutrient levels were equal to the intake target ratio (Simpson and Raubenheimer 2001). At protein:carbohydrate ratios lower than optimal, food intake was significantly reduced, while at protein:carbohydrate ratios higher than optimal, nitrogen utilization efficiency was reduced. Tannic acid therefore seems most effective in reducing feeding efficiency in conditions of imbalanced, i.e. low protein:carbohydrate food supply. Nitrogen deposition can result in higher protein:carbohydrate or lower protein:carbohydrate ratios, the latter especially in soils poor in nutrients. The interaction of nitrogen deposition, soil type, and plant defence strategy can thus affect the outcome of plant-herbivore relationships, but only under conditions of suboptimal food quality.

Couture et al. (2010) found an 18% decrease in cardenolide concentrations in N-fertilized *Asclepias curassavica*, a result of increased plant growth relative to cardenolide synthesis under N fertilization, which seems to occur regularly in this species group (Agrawal et al. 2012). Elevated latex cardenolide concentration in Asclepias has been found to reduce monarch butterfly *Danaus plexippus* growth rate (Tao and Hunter 2012), even though this species is known to have evolved mechanisms to sequester these compounds (Agrawal et al. 2012). These studies indicate that increased plant growth due to nitrogen deposition may lower the effectiveness of plant herbivore deterrent strategies via dilution effects and/or reduced investments of C into allelochemicals, and can thus lead to an increase in food quality for specialist consumers.
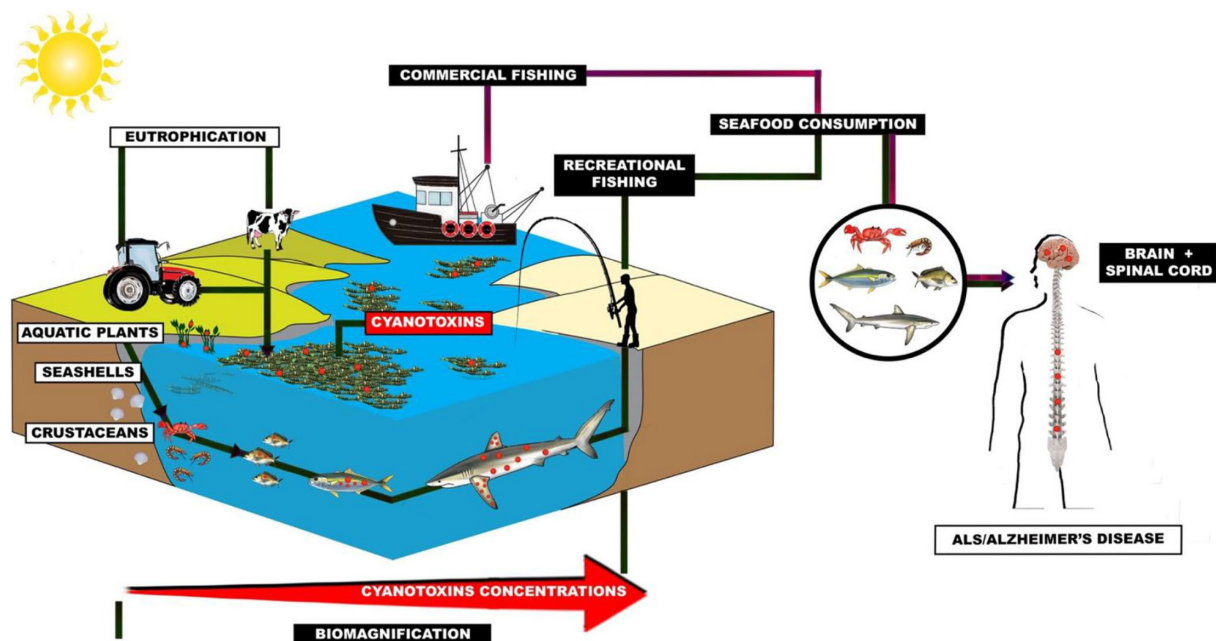
Figure 15*. Eutrophication driven increase in cyanotoxin and non proteionomic amino acid producing algal blooms and bioaccumulation pathway, leading to increased risk in neurodegenerative disease in humans. Figure taken from Dunlop and Guillemin (2019).*

Figuur 15*. Door eutrofiëring veroorzaakte toename van cyanotoxine en niet-proteïonomisch aminozuur producerende algen en bioaccumulatieroute, leidend tot een verhoogd risico bij neurodegeneratieve aandoeningen bij de mens. Figuur uit Dunlop en Guillemin (2019).*

2.6.3 Summary conclusions

The effect of N deposition on allelochemical based producer-consumer interactions depend on the nature of nutrient limitation, the chemical composition of the allelochemical involved (mostly whether the compound contains N or not) and the feeding strategy (specialist vs generalist) of the consumer. For N containing compounds, alleviation of N limitation in producers often results in an increase of N-containing allelochemicals, thus potentially reducing nutrient quality for consumers.

In contrast, fully C based allelochemicals often (but not always) show reduced concentrations under increased N supply, due to dilution effects resulting from increased growth and/or reduced production of these compounds resulting from increased C assimilation rates in plant tissue. Drought stress is often found to interact or act as prerequisite for reduced C based allelochemical concentrations under high N supply.

The consumers response to an increase or decrease of allelochemicals depend on the working mechanism of the allelochemical (phytotoxin or antifeedant), feeding strategy and the general physical and physiological adaptations used by the organism in obtaining an adequate amount of nutrients. A general rule of thumb is that the effect size of consumers response to increased or reduced levels of allelochemicals is greater in generalist consumers than in specialist consumers, and smaller in consumers adapted to low nutrient quality than consumers adapted to feeding on plant tissue with high nutrient value.

We hypothesize that N deposition mediated changes in producer allelochemicals will result in stronger responses of generalist herbivores than specialist consumers. Species specialized in feeding on plants using N containing allelochemicals will only moderate decline in fitness, while generalist species will show stronger fitness effects. Generalist species will however show stronger fitness increases when feeding on plants utilizing a phytotoxic strategy that are naturally growing under N limited conditions, possibly resulting in increased population growth and or increased occurrence of plague events. For plants that mostly produce antifeedant allelochemicals, these differences will be less pronounced and both specialists and generalist will show similar positive responses to decreases in plant allelochemicals.

2.7 Adaptations to low or inadequate food quality

Also under natural conditions, consumers generally have to cope with producer food quality that is suboptimal at best. During the course of evolution, consumers have developed many different adaptations and strategies to cope with inadequate food quality. These adaptations help the organism in question in order to maximize growth efficiency, survival and population persistence. The type of adaptation employed by consumers may thus also play an important role in the response outcome when N deposition leads to an altered nutritional quality of producer tissue. Some of these adaptations are already discussed in the earlier paragraphs of this chapter, in which case, the are regarded as especially relevant in the context of the nutritional framework discussed (e.g. compensatory and complementary feeding strategies in het NG framework; section 2.4, host plant specialism vs generalism in ES, NG and allelochemical frameworks; sections 2.2, 2.4 and 2.6). Many other types of adaptations that are (at least partially related to coping with inadequate food quality) are however not discussed. In this section, we provide a general overview of consumers evolved adaptations to overcome inadequate food quality, as sufficient prior knowledge on a consumers nutritional strategy may often be required in either devising hypotheses or interpreting effect outcomes of producer-consumer interactions to N deposition mediated changes in producer quality. For reasons of completeness, the adaptations discussed may show some overlap with earlier sections in this chapter. A general overview of (evolutionary) adaptations to cope with low food quality is also given in **Table 6**.

2.7.1 Trophic level and width: generalism, specialism, omnivory and carnivory

Many consumers obtain a broad diet comprising several different food types, which may often even encompass different trophic levels (in which case, these are referred to as omnivores). Nutrient composition and flow may differ considerably between species and/or trophic levels, so dietary mixing may reduce the degree of shortages in nutrients (see also paragraphs 2.2 and 2.4). This dietary mixing comes with a cost in terms of lower utilization efficiency of individual food types: a generalist feeder will be less adapted to maximizing growth efficiency on a single species, and will perform poorer on producers high in phytotoxic allelochemicals than specialist species. For generalist feeders, chronic inadequacies in food intake will be mostly driven by a decline in species diversity and hence a decrease in the ability to compensate for inadequate food intake. An

illustration of this can be found in recent human history: Corn *(Zea mais)* grains are naturally poor in the essential amino acid tryptophan (Oser 1965). During the economic depression of the nineteen twenties, many people were forced to feed solely on corn as they were economically restricted from other food sources. This narrow diet caused thousands of deaths due to niacin deficiency as tryptophan is used as a precursor of this semi-essential B-vitamin.

The obvious opposite consumer strategy is to specialize on a narrower diet. Many herbivores show specialization on a given group (i.e. grasses), small species set (i.e. species from one plant family) or a single host plant. Although not solely driven plant nutrition and/or plant defence co-evolution pathways (van Genderen et al. 1996, Agrawal et al. 2012) (i.e. competition reduction can also be a selective pressure), host plant specialization enables species to maximize the nutrient acquisition efficiency on the typical, often quite predictable nutritional status of the given food species. Such species will typically have metabolic and/or symbiotic strategies aimed at dealing with the inadequate nutrients in the food. For a food specialist (next to loss of the host plant), changes in nutrient content of the host plant will therefore be much more likely to have major fitness effects than in more generalist species (see also section 2.2 and 2.4), which as a general rule may also apply to inadequate micronutrient.

Predation and/or omnivory can also be regarded as a specialist strategy focused on release from (micro)nutrient deficiencies, which is often most pronounced in top predators, as animal prey usually accumulated sufficient (micro)nutrients to also satisfy the nutritional requirements of the predator. *Felidae* (cats) are an example of this strategy, as they lost the capabilities to use precursors for vitamin A and essential fatty acids (Twining et al. 2016) in their food to obtain the active components. This strategy may then be hazardous if prey food quality for these micronutrients deteriorates significantly.

2.7.2 Physiological adaptations

Physiological adaptations encompass evolved strategies that maximize the rate of uptake of the most limiting nutrient, by increasing the synthesis (and hence concentration) of enzymes involved in extracting these nutrients (Elser et al. 2010b), or through the evolution of enzymes with increased affinities for the limiting nutrient (often at the expense of enzyme capacity). Generalist consumers often employ a high enzyme concentration strategy, whereas food specialist often employ a high affinity enzyme strategy (Mattson and Scriber 1987). Therefore, generalists often show stronger growth responses to increases in nutrient concentrations than specialists. Specialists on the other hand are often able to utilize food items lower in the limiting nutrient than generalists, but show less strong responses to increased concentrations of the limiting nutrient. Larger body size, and/or lowered basal metabolic rate (BMR) are often associated with feeding on poor quality food (Mattson and Scriber 1987), since BMR decreases with body size, and larger organisms are also more capable to digest fiber-rich food.

Micronutrient deficiencies can be circumvented by the capability to use (a wider array of) precursors to metabolize the required micronutrient, but this capability is highly species dependent. An example is in the metabolism of vitamin A using carotenoid precursors. Animals differ in the type of precursors they can modify into active forms of the vitamin (retinol and retinoic acid). In humans, beta-carotene is a common precursor, but other animals may for example also use lutein or zeaxanthine (Brenes-Soto and Dierenfeld 2014). Other animals such as wild cats, do not eat vegetable matter and fully rely on the active vitamin in their animal prey.

If either food quality or the requirements in terms of micronutrients varies over time, a viable strategy is to store micronutrients or spare them from catabolic breakdown. In the vertebrate body, this is usually possible for fatty acids, fat soluble compounds, amino acids, and some elements (e.g. skeletal calcium), but typically not for water soluble (B) vitamins. Fatty acids are typically used to fuel the animal body, but some of these are essential micronutrients, which an animal would want to keep for more important physiological functions. Indeed, fatty acid combustion is arranged to spare the essential micronutrients among the total array of fatty acids.

A process in which demands for micronutrients are particularly high is for example egg laying in birds. In a short amount of time the eggs must be supplied with all nutrients that would last the incubation period and the first hours after hatching. Depending primarily on the ratio of body size versus total egg volume of the clutch, birds may draw more or less on their body reserves in clutch formation. Large birds that lay few eggs (capital breeders: e.g. the large eagles) can produce their eggs from stored nutrients and B vitamins in circulation. In contrast an income breeder, for instance a small songbird that produces a clutch that equals her own body size (or more) relies largely on food intake during egg laying. Birds are sensitive to amino acid deficiencies during reproduction, because the amino acid requirements of avian eggs differs significantly from the make-up of the female body tissue proteins (Van den Burg in press). So, eggs are still prone to amino acid deficiencies, under conditions in which adult birds are not affected. Egg laying female birds can compensate amino acid deficiencies in their food by recruiting missing amino acids from their breast muscles (Houston et al. 1995, Selman and Houston 1996, van den Burg 2009). This process requires large quantities of breast muscle to be broken down in order to gain a relatively low amount of the essential amino acids stored in the muscle tissue.

2.7.3 Microbial symbiosis

An widely (if not obligately) used adaptation to overcome inadequate food quality is through symbiosis with gut microbes, helping the organism to digest and assimilate low (i.e high C:N) food types. Mycetocyte symbiosis in insect orders enables these to digest food with low protein:carbohydrate ratio (Simpson and Raubenheimer 1993), thus substantially reducing dietary N demand. An even stronger symbiotic relationship is found in phloem sap feeding aphids, which obtain essential amino acids from *Buchnera* endosymbionts, that are present in specialized cells called bactericocytes in the aphid body. These convert ingested and nitrogenous waste products into amino acids, with carbohydrate delivered by the aphid in return (Douglas 1998). Interestingly, Aphid NG studies (Abisgold et al. 1994, Simpson et al. 1995) only found behavioral intake regulation for carbohydrates, but not for amino acids.

Animals that use bacteria in their foregut and/or gut to digest poor quality food compounds may also use bacterial protein in order to eliminate amino acid deficiencies, as bacteria typically synthesize the entire suite of essential amino acids. Similarly, these bacteria can also provide in many of the essential vitamins. Animals using these strategies are typically herbivores or species living of dead wood and include ruminants (McDonald et al. 1995), but also many insects use gut microbes for this purpose (Chapman 1998). Virtually all animals have bacteria in their digestive system, but this does not warrant the use of bacterial protein. In order to be able to utilize bacterial amino acids, specific digestive enzymes must be produced by the consumer. This is not the case in, for example, humans and wild boar *Sus scrofa*. (Bergen 2015). Some bird groups also take advantage of bacteria to aid digestion, such as pheasants, grouse, and ducks. These birds have large caeca up to a combined length equaling the small intestine size. Intriguingly, other birds, such as owls, co-evolved the same organs to harbor bacteria that recycle uric acid produced by the kidney (Denbow 2000). Bacterial amino acids are harvested as birds release proteolytic enzymes in the caeca to aid the breakdown of microbial protein. In conclusion, consumer species with strong microbial symbioses (i.e. specialized cell structures, gut morphology, specific enzyme activity) are less vulnerable to low N content of food or to specific amino acid deficiencies.

2.7.4 Morphological and behavioral adaptation

As lower nutrient quality also results in lower potential relative growth rate, organisms adapted to poor quality food generally developed several behavioral (e.g. hiding) and physical adaptations (e.g. development of spines) to counteract the longer exposure time to predators and parasites. Ungulate herbivores are also known to forage selectively for high Na sources (Borer et al. 2019) and osteophagy and even carnivory have been observed in response to P shortage (Wallis de Vries 1998, Gambín et al. 2017, Dixon et al. 2019). Herbivorous invertebrates are also known to be able to increase gut volume under conditions of poor nutrient quality and in increasing food residence

time (Yang and Joern 1994, Raubenheimer and Bassil 2007), or have a high relative gut volume by default, in case the organism specializes on highly imbalanced food items (i.e. ruminants).

Behavioral thermoregulation, larger body size, dense hairs, dark body coloration, construction of web-tents and other thermally favorable structures are all adaptations enabling insects to increase their body temperature well above ambient levels. This also enables poikilotherms to increase respiration rates and hence increase C losses through respiration (Trier and Mattson 2003). Homeothermic consumers on the other hand often exhibit adaptations that enable these organisms to lower their body temperature, since these organisms will have higher fitness when they are able to reduce basal metabolic rate as high enzyme activity is the default state in homeotherms.

2.7.5 Summary conclusions

Consumers have developed a wide array of adaptations to cope with inadequate food quality. These adaptations however cannot be regarded in isolation, but as an often inseparable part of an organisms life history tactic that may reduce or increase an organisms susceptibility to changed nutrient quality. Using this paradigm, adaptations provide benefits for an organism to cope with inadequate food quality that equal or surpass the expense and constraints associated with it. Also, a given adaptation often has facultative or even obligate interrelationships with other adaptations. For instance, gut volume and gut morphology is often associated microbial symbiosis in the organism. Which is in turn at the expense of a higher minimum required body size for reproduction, longer development time and increased risk of predation during the development period.

Within the framework of N deposition mediated changed nutrient quality, the specific life-history tactic employed by an organism thus ultimately determines the degree and direction of the response of (populations of) the organism to this change in nutrient quality. In studies specifically aimed at describing the effect of N deposition on specific consumer species, prior knowledge on the strategy used by the species in acquiring adequate amounts of nutrients may be crucial to devise hypotheses and/or interpret result outcomes of N-manipulation experiments. Studies aimed at the effect of N deposition on consumer community composition can also benefit from prior knowledge or a priori hypotheses based on different nutritional life history tactics. Using such a trait or life history tactic based approach in field studies may thus provide a mechanistic understanding of observed effects in community composition, and may be pivotal in explaining contrasting responses of species or species groups to increased N deposition. Without aiming for completeness, we provide three exemplary hypotheses based on traits and life history tactics.

Depending on the type of changed nutrient quality, we hypothesize that following increased N deposition:

1. Changed N:element ratios will result in:
   a. no increase or some decreased fitness of species employing a LHT aimed at maximizing uptake efficiency of the limiting nutrient using a slow-growth tactic: large-bodied, low BMR species with high relative gut volume, high dependence on gut microbial symbiosis with associated gut morphology.
   b. an increase of fitness of species employing a strategy aimed at maximizing uptake volume of the limiting nutrient using a medium to fast growth tactic: small to medium bodied, polyphagous species with high relative gut volume and high enzyme production; low dependence on gut microbial symbiosis or no specific gut morphological adaptations for microbial symbiosis.
   c. a decrease of fitness of species employing a strategy aimed at maximizing uptake efficiency of the limiting nutrient using a fast growth tactic: small to medium bodied, monophagous (selective feeding) species with low relative gut volume and high affinity enzyme production; low dependence on gut microbial symbiosis and no specific gut morphological adaptations for microbial symbiosis.

2. Changed amino acid concentration will result in:
    a. A decrease in fitness of species using a strategy with low storage capacity for amino-acids or high dependence on muscle tissue, without using endosymbionts in amino-acid acquisition
    b. No response in fitness of species with high storage capacity for amino acids or maintaining endosymbiotic relationships with amino-acid producing bacteria (the latter might even increase as a result of overall higher food N content)
3. Reduced (micro)elemental concentration will result in:
    a. No decrease in species using other types of elemental resources (pica-behavior; drinking mud water or urine) or species specifically supplementing food with Ca rich (vertebrates, mollusks, millipedes, woodlice) prey, and
    b. A decrease in species not having evolved these behavioral mechanisms.

Table 6. Overview of evolutionary adaptations of consumers to cope with suboptimal food quality, summarizing the benefits and constraints associated with given traits. Under "other remarks", links with other adaptations or examples are given, when appropriate. Adaptations derived from Mattson (1980), Mattson and Scriber (1987), Raubenheimer and Simpson (1993), Simpson and Raubenheimer (1993), Yang and Joern (1994), Elser et al. (1996), Douglas (1998), Elser et al. (2000a), Denno and Fagan (2003), Raubenheimer and Bassil (2007), Hillebrand et al. (2009), Setamou et al. (2016), Shukla et al. (2016), Filipiak et al. (2017).

Tabel 6. Overzicht van evolutionaire aanpassingen van consumenten tegen suboptimale voedselkwaliteit, met een samenvatting van de voordelen en beperkingen van deze eigenschappen. Onder "Other remarks" worden, waar van toepassing, koppelingen naar andere aanpassingen of voorbeelden gegeven. Aanpassingen afgeleid van Mattson (1980), Mattson en Scriber (1987), Raubenheimer en Simpson (1993), Simpson en Raubenheimer (1993), Yang en Joern (1994), Elser et al. (1996), Douglas (1998), Elser et al. (2000a), Denno en Fagan (2003), Raubenheimer en Bassil (2007), Hillebrand et al. (2009), Setamou et al. (2016), Shukla et al. (2016), Filipiak et al. (2017).

Adaptation	Benefits	Constraints	Other remarks
Polyphagy	Increased nutritional space by utilizing generic assimilative traits (large body mass, increased enzyme synthesis) utilize 'compensatory feeding' with plant species having different stoichiometry	Lower NAR due to costs associated with adapting to different food composition Metabolic costs associated with movement	
Monophagy	Opportunity to optimize assimilation rates of nutrients by fine tuning behavioral and physiological traits. Selective feeding on richest plant parts	Low plasticity with regards to intra-species variation in nutrient quality. Requires synchronization with host plant seasonal growth pattern	
Compensatory feeding	Increase consumption rate to meet nutritional requirements.	Sensory information of nutrient content and nutrient demand must be available. Increased food intake also leads to increased waste handling.	
Complementary feeding (facultative polyphagy)	Utilize food items differing in nutrient ratio in order to complement nutrient shortages	Complementary food items must be available. Often requires higher mobility (i.e. results in higher metabolic costs)	Generalists have broader nutritional geometry than specialists, as the change of encountering complementary food is larger in the former group, and C is used more for fuel for locomotion (in high dispersive generalists)

Table 6. (continued)

Adaptation	Benefits	Constraints	Other remarks
Facultative carnivory	Occasionally consume insect or animal tissue to meet nutrient demands		Theoretically, many herbivorous species groups should easily be able to use this strategy.
Obligate carnivory	Switch from herbivory to carnivory during parts of the life cycle to meet nutrient demands	Herbivorous building plan not well- suited for predation	Ant brood parasitism by larvae of many Lycaenid butterflies is a well-known example
Intraguild predation	Consume prey items which better match consumer C:N ratio	Increased mortality risk when hunting predacious prey items	
Reduced basal metabolic rate	Lower daily nutrient requirements (optimizing BMR to maximum ATP synthesis)	Longer development time	
Reduced Relative Growth Rate	Lower requirement for proteins (N) and protein synthesis (ribosomal P).	Longer development time	Allometric relation between body size and RGR: small species have higher RGR than larger species. Induces evolution of larger body size
Increased development time	Lower daily nutrient requirements. Lower BMR. Higher assimilation efficiency (at the cost of assimilation speed)	Prolonged exposure time to predation and parasitism. Trade-off between optimal body size and development time	Increased investments in traits reducing predation (shelter, spines, poisons, etc).
Increased food intake	By processing more bulk food, enabling to acquire more nutrients (see also compensatory feeding)	Requires large (relative) stomach and gut volume; reduces dispersal capability	
Increased food processing time	Increasing total nutrient uptake by extending the gut passage period of ingested food	Requires large (relative) stomach and gut volume; reduces dispersal capability	Often in symbiosis with gut microorganisms. Also includes ruminant and auto-coprophagous feeding strategies
Increasing enzyme concentration	Higher assimilation speed and uptake capacity of (limiting) nutrient	Metabolic costs associated with increased enzyme synthesis	Temperature and thermoregulatory adaptations
Increasing enzyme affinity	Higher assimilation efficiency (and hence speed) of limiting nutrient.	Low responsiveness to changes in nutrient concentration	Temperature and thermoregulatory adaptations
Microbial symbiosis	Use (endo)microbial symbionts for acquiring nutrients and in providing essential nutrients to the herbivore host. Enables to feed on low nutrient food items (especially N).	Adaptations in gut structure and volume, carbohydrate supply to symbiont	
Increasing excretion rate and/or efficiency	Enhanced excretion of non-limiting elements back into the environment.	May cause further imbalance in food through consumer driven resource recycling. Metabolic costs associated with increased excretion	

Table 6. *(continued)*

Adaptation	Benefits	Constraints	Other remarks
Increased body size	Lower BMR, RGR, increased gut content, better thermoregulation	Increasing body size increases development time resulting in higher risk of predation before successful reproduction is achieved	
Thermoregulation: optimizing enzyme efficiency	Higher assimilation efficiency (and hence speed) of limiting nutrient	Basking required increases predation risk	
Thermoregulation: lowering body T to reduce respiration and BMR	Lowering BMR when not assimilating conserves energy and nutrients		
Supplementary feeding; "pica behavior"; Osteophagy	Supplementing limiting nutrient intake by consuming nutrient rich clays, mineral rich water, animal excrements, animal bones, urine or tree sap	Waste products are also consumed	Apparent limitation in an organism might be easily overcome by these evolved behavioral mechanisms

3 Resumé and general conclusions

3.1 C:N:P elemental stoichiometry

- The relative availability of the major elements (i.e. elemental stoichiometry) rather than concentration of one of the single elements C, N or P determines individual and/or population growth rate, reproduction and survival, which are optimal at the threshold elemental ratio (TER) of given nutrients.
- Producers exhibit greater stoichiometric plasticity than consumers, and generally have higher C:N as well as N:P stoichiometric ratios than consumers.
- Shifts in producer C:N:P ratios may result from changes in N (and P) availabilities. Under increased N deposition, producer C:N ratios are likely to decrease, but N:P ratios are likely to increase, possibly leading to enhanced or shifts towards P limitation.
- Under increasing N load, consumers with relatively low C:N TERs are expected to be more often positively affected by N deposition, but at the same time, consumers with relatively low N:P TERs are expected to be more often negatively affected by N deposition.
- Whether, to what extent and in which direction increased N deposition mediated shifts in producer C:N:P ratios alters the performance of consumers depends on the type of ecosystem (oligotrophic vs eutrophic), producer species as well as consumer species.
- For consumer species, the C:N:P TER is determined by physiological functioning, phylogeny, ontogeny, behavioral adaptations and other species traits, such as feeding mode, dispersal capability and host plant and/or food type specialization.
- For terrestrial invertebrates, there exists supporting evidence for the hypotheses that 1) specialists occupy a narrower stoichiometric niche than generalists, 2) specialists tend to feed on host plant species that are more similar in nutrient stoichiometry, 3) C:N:P stoichiometry in consumers is scaled allometrically and 4) amongst insects Lepidoptera and Diptera are most vulnerable to changed producer N:P ratio, due to their relatively low body tissue N:P ratio compared to other groups.

3.2 Other elements and trace metals

- Metal ions and trace elements play an important role in enzyme functioning, cellular functioning and homeostasis and are essential for organismal functioning in producers and consumers.
- Ionomic mismatch between producers and consumers seems to occur frequently in the natural environment and the degree to which mismatches occur can be affected by nitrogen deposition via eutrophication or acidification pathways.
- Reports of elemental limitation in consumers are mostly confined to Ca deficiencies in avian predators. For other groups of organisms the scarce studies are confined to correlative studies, but are receiving more scientific interest recently which suggest a greater role of elemental mismatch in consumer species population dynamics and individual performance.
- We conclude that there exists a knowledge gap in the effect of elemental composition on consumer performance and the effect of increased N deposition on producer-consumer interactions. Especially the effect of lowered elemental concentration of Na, but also K, Mg and trace elements as Cu, Se have been reported to be of direct or indirect importance in determining consumer fitness, but for all the elements, the generality of limitation is far from clear and requires extensive further study.

3.3 Proteins and carbohydrates

- Proteins, carbohydrates, fatty acids and lipids can be considered as the real currencies of the elemental nutrients N (proteins) and C (carbohydrates, fatty acids and lipids) that consumers need for growth.
- N and C containing metabolites made by producers (amino acids, fatty acids), are often essential for the consumers. For consumers, the carbohydrate:protein ratio is a better measure of macronutrient quality than producer C:N ratio.
- The ratio in which carbohydrates and proteins exist in food items often determine the nutritional quality. Homologous to ES theory, these ratios are not linearly linked to either higher carbohydrate or protein content, but instead an optimum ratio at which maximum growth can be achieved exists (referred to a species 'intake target').
- Consumers are able to adapt their consumption effort and behavior to cope with suboptimal food quality: when offered food of general low nutritional value by increasing the ingestion rate (compensatory feeding) and when offered food of suboptimal nutrient ratios by selectively feeding on complementary food items, when available (complementary feeding).
- The degree to which consumers balance food intake depends on ontogeneny and life-history traits: larval and nymphal stages balance their intake to such a degree that it matches the intake target as close as possible (closest distance optimizers). Adult life stages and highly mobile generalist species in particular often over-ingest a given nutrient to some degree (fixed proportion optimizers).
- The nutrient intake strategy used by a consumer thus determines the sensitivity to N deposition mediated changes in protein:carbohydrate ratios in producers.
- Homologous to ES theory, N deposition mediated changes in protein: carbohydrate ratios would affect low-dispersing host-plant specialists to a much greater degree than high-dispersing host-plant generalists. The larval or nymphal stage is hypothesized to be most vulnerable to such changes.

3.4 Essential micronutrients

- Micronutrients are not well-studied in consumers, and even less in the perspective of increased N deposition.
- Livestock, veterinary and human studies warrants the conclusion that several pathways for micronutrient limitations may exist. From the suite of existing micronutrient limitations which cause harm to animals, only a few can be related to N deposition in theory or in practice.
- In aquatic communities, micronutrient driven shifts in producer-consumer interactions are often caused by eutrophication and often related to shifts in the composition of algal primary producers, ultimately resulting in fatty acid and/or deficiency in consumers. In terrestrial ecosystems, micronutrient deficiencies generally result from intraspecific changes of micronutrient availability in producers.
- Changes in producer micronutrient levels can be directly caused by N-enrichment (imbalance in amino acids) or by acidification via reduced uptake of elements essential in micronutrient synthesis (i.e. Zn for vitamin A, Co for vitamin B12).
- Consumer species that are especially at risk to encounter micronutrient deficiencies are either top-predators (in case of amino acids, riboflavin, thiamin) or first order consumers (in case of fatty acids, vitamin A and vitamin B12).

3.5 Antifeedants and phytotoxins

- For N containing compounds, alleviation of N limitation in producers often results in an increase of N-containing allelochemicals, thus potentially reducing nutrient quality for consumers.

- C based allelochemicals often (but not always) show reduced concentrations under increased N supply, due to dilution resulting from increased growth or reduced production of these allelochemicals.
- Drought stress is often found to interact or act as prerequisite for reduced C based allelochemical concentrations under high N supply.
- The effect of N deposition on allelochemical based producer-consumer interactions depend on the nature of nutrient limitation, the chemical composition of the allelochemical involved (mostly whether the compound contains N or not) and the feeding strategy (specialist vs generalist) of the consumer.
- In general, consumers response to increased or reduced levels of allelochemicals is greater in generalists than in specialists, and smaller in consumers adapted to feeding on tissue of low nutrient quality.
- We hypothesize that N deposition mediated changes in producer allelochemicals will result in stronger (either positive or negative) responses of generalist herbivores than specialist consumers. Species specialized in feeding on plants using N containing allelochemicals will only moderate decline in fitness, while generalist species will show stronger fitness effects. Generalist consumers will more often encounter fitness increases when feeding on plants that are naturally growing under N limited conditions.

3.6 Consumer adaptations to inadequate food quality

- Consumers have developed a wide array of adaptations to cope with inadequate food quality. Specific adaptations often have facultative or obligate interrelationships with other characteristics or adaptations, depending on the species nutritional niche. This set of adaptations thus forms the organisms life history tactic, which in turn determines an organisms susceptibility to changed nutrient quality.
- In studies aimed at investigating N deposition effects on specific consumer species or consumer community composition, prior knowledge on the strategy used by the species in acquiring adequate amounts of nutrients may be crucial to devise hypotheses and/or interpret result outcomes of N-manipulation experiments.
- Using a trait or life history tactic based approach in field studies may thus provide a mechanistic understanding of observed effects in community composition, and may be pivotal in explaining contrasting responses of species or species groups to increased N deposition.

3.7 General conclusions

Nutritional frameworks in ecology vary greatly in degree of complexity. A general rule is that under increasing complexity, a decreasing amount of relevant literature exists. Ecological stoichiometry, nutritional geometry and the role of antifeedants and phytotoxins have received most attention in ecological studies, whilst effects of micronutrients are hardly studied in an ecological context. The ecological relevance of (trace) element supply is only recently receiving (renewed) increased scientific interest. With this varying degree of understanding in mind, still some general conclusions and hypotheses can be drawn when applying these frameworks to changed producer quality due to increased N deposition.

Ecological stoichiometry and nutritional geometry frameworks have both postulated the hypothesis that specialist occupy narrower 'nutritional niches' than generalist feeders. Under increased N deposition, changed CNP stoichiometry or protein:carbohydrate ratios may more often lead to reduced fitness of host plant specialists than generalists. Moreover, this reduced fitness should occur most markedly in relatively small, low dispersing univoltine insect species. In contrast, N deposition mediated increased consumer fitness is more likely to occur in larger, well dispersing generalist species, especially when increased N deposition concomitantly leads to a reduction in C based antifeedants or phytotoxin concentrations in producers.

Nutritional geometry studies also made clear that different species have distinctly different intake targets, both in absolute amounts of required nutrient intake and in nutrient (protein:carbohydrate) ratios. Phylogenetic constraint also seems to partly determine a species 'nutritional niche'. Species belonging to taxonomic lineages with relatively low N:P stoichiometry (Diptera and Lepidoptera) are considered most vulnerable to increased N deposition and associated changes in producer N:P stoichiometry. It is likely that for other (trace) elements, phylogenetic constraint also determines consumer requirements, but as of yet this has not been studied.

Such filtering mechanisms on species traits as well as taxonomic lineages would ultimately result in a reduction of total biodiversity in a given ecosystem. When nutrient ratios change as a result of increased N availability, species encountering changes towards the optimum of their nutritional niche will benefit while species that encounter the opposite will suffer. The ecological significance of micronutrients is the least well-studied topic in ecology and hence general rules or hypotheses are cannot be drawn from current literature. Of the micronutrients reviewed here, some deserve more scientific study in an ecological context, especially the role of fatty acids, vitamin A and vitamin B12 in shaping first order consumers population dynamics and of amino acid composition, riboflavin and thiamine in those of top-predators.

Ultimately, chronically increased N-deposition above critical levels may result in an overall decrease in consumer biodiversity as a result of the filtering effect imposed on consumer populations. The general underlying mechanisms summarized here are greatly modified by the particular ecological niche occupied by the specific organism in question (e.g. terrestrial invertebrates vs aquatic invertebrates), and by other species traits such as feeding modes (e.g. grazers vs browsers vs sap suckers vs filter feeders), (endo)symbiotic relationships, body plan (e.g. higher Ca requirements of vertebrates), physiology and behavioral adaptations. A mechanistic understanding of the effects of N deposition on producer quality is therefore not only depending on sufficient knowledge of the effects of N deposition on soil chemistry and producer quality (in other words: lowered C:N ratio is not in every case beneficial for consumers, although in many cases it would), but also on the life history strategy of the consumer species. In studying the effects of N deposition on producer quality in field situations, a trait or life history tactic based approach is crucial to devise a priory hypotheses on consumer response and in devising experiments with species having contrasting life history tactics. Such studies may be highly beneficial in providing a mechanistic understanding of N deposition effects on consumer community composition, and in predicting the response of particular species or species groups to increased N deposition.

Part 2: N deposition effects on the environment, producer quality and consumer performance

4 Nitrogen deposition effects on the environment

4.1 Global increase of N deposition and acidification rate

N is the limiting nutrient for plant growth in many natural and semi-natural ecosystems, especially in oligotrophic and mesotrophic habitats. The severity of the impacts of atmospheric N deposition depends on a number of factors, of which the most important are (numbers not being a ranking): (1) the duration and total amount of inputs, (2) the chemical and physical form of the airborne N input, (3) the intrinsic sensitivity of the plant and animal species present, (4) the abiotic conditions, including climate, and (5) the past and present land use or management. Acid neutralising capacity (ANC), soil nutrient availability, and soil factors that influence the nitrification potential, N immobilisation and denitrification rates, are especially important. As a consequence, different ecosystems show high variability in sensitivity to atmospheric N deposition (Bobbink et al. 2010a). Despite this highly diverse sequence of events, it is possible to generalize some main effect pathways. A schematic overview of the potential sequence of events is given in **Figure 16**.
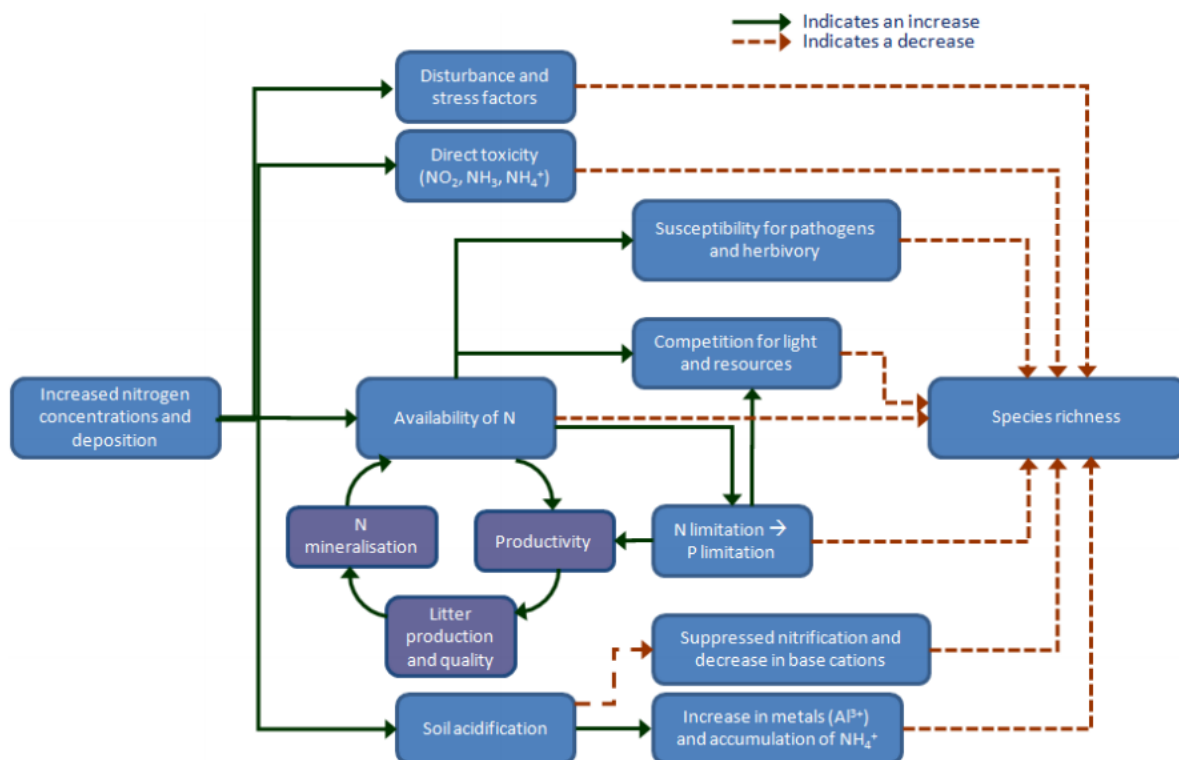
Figure 16. *Schematic overview of the main impacts of increased N deposition on ecosystems. Stress is considered to occur when external constraints limit the rate of dry matter production of the vegetation, whereas disturbance consists of mechanisms which affect soil and plant biomass by causing its partial or total destruction. From Bobbink et al. (2010a), chapter 2.*

Figuur 16. *Schematisch overzicht van de belangrijkste effecten van verhoogde N-depositie op ecosystemen. Stress wordt beschouwd op te treden wanneer externe beperkende factoren de droge stofproductie van de vegetatie beperken, onder verstoring worden mechanismen verstaan die als gevolg hebben de biomassa van de bodem en de plant negatief te beïnvloeden. Uit Bobbink et al. (2010a), hoofdstuk 2.*

Despite this diverse sequence of events, the most obvious effects of increased N deposition are eutrophication with significant changes in the N cycle, soil acidification, and decreased biodiversity of plants and animals (Bobbink et al. 1998, Bobbink et al. 2010a, Bobbink et al. 2010b).

4.2 Terrestrial ecosystems

4.2.1 Eutrophication

Increased N deposition results in an increase in the availability of inorganic N in the topsoil. This gradually leads to an increase in plant productivity in N-limited vegetation, and thus to higher annual litter production and litter with (slightly) higher concentrations of N. Because of this, N mineralisation will also gradually increase, which in turn may increase plant productivity. Increased N deposition essentially creates a positive feedback loop, as higher N mineralisation leads to higher N uptake, higher production and higher litter N. The rate of N cycling in oligotrophic ecosystems can thus be substantially increased, although the response time to increased N inputs can be long in highly organic soils (i.e. with high C:N ratios), or any other soil type with large potential N sinks. The decomposition of more difficult substrates (i.e. soils with high litter C:N ratios or litter with high lignin content) can however also be reduced as most Basidiomycetes that breakdown lignin respond negatively to N addition. This may lead to disbalances in systems with tightly coupled N cycles. Irrespective of these potential negative feedback loops, the general pattern that is observed is a significant decrease in soil C:N ratio with longer-term increased N deposition (De Vries et al. 2015).

Above a certain level of primary productivity, local plant species diversity declines as production increases. Observational studies across N deposition gradients, and many N-addition experiments, demonstrate this effect in the long term. Competitive exclusion ('overshading') of characteristic species of oligotrophic or mesotrophic habitats occurs in the presence of relatively fast-growing species, with rare species at low abundances being especially at risk (**Figure 16**) (e.g. Bobbink et al. 1998, Dise et al. 2011). This drastic change in species composition can additionally influence the nutrient availabilities in the soil, because of different litter quality of the new dominants (e.g. grasses) compared to the original vegetation (e.g. small forbs or dwarf shrubs), and thus affected decomposition rates. When N is no longer limiting, plant growth becomes restricted by other resources, such as phosphorus (P), potassium (K), magnesium (Mg), or water availability. In this situation, the productivity of the vegetation will not increase any further with continuing increases in N input. However, N concentrations within the plants do tend to increase when N availability continues to increase, thus lowering the N to other nutrients ratios. This may affect the palatability of the vegetation for herbivores.

It is concluded that soil N availability increases with higher N loading from the atmosphere and in the longer term soil C:N ratio will significantly decrease (e.g. Bobbink et al. 2010a, Velthof et al. 2011). At the same time, soil N:P ratio has consistently been reported to increase (Penuelas et al. 2020). Such shifts in the balance of the major biological elements in soil has also been found to influence the nutritional balance of primary producers in terrestrial ecosystems, affecting community structure and ecosystem functioning (Penuelas et al. 2020) which could ultimately cascade as well to higher trophic levels (see chapters 0 and 0 in this report).

4.2.2 Acidification

Soil acidification is defined as the loss of acid neutralizing capacity (ANC) and may lead to a decrease in soil pH. Changes in pH are dependent on the buffering capacity of the soil (e.g. Ulrich 1983, 1991). Acidifying compounds (N and S) deposited on calcareous soils (including substrates of young moraine regions) at first will not change soil acidity. In these soils HCO_3^- and Ca^{2+} ions leach from the system, but the pH remains the same until almost all of the calcium carbonate has been depleted. In soils dominated by silicate minerals (pH 6.5-4.5), buffering is taken over by cation exchange processes of the soil adsorption complexes. In this situation, protons are exchanged with Ca^{2+} and Mg^{2+}, and these cations are leached from the soil together with anions, mostly nitrate or sulphate (Blume et al. 2016). Because of the restricted capacity of this buffering system, soil pH

will soon start to decrease. However, in mineral soils with a large cation exchange capacity (CEC) and high base saturation, this soil buffering system may continue to function for several decades, even at relatively high inputs. At low pH (< 4.5-5.0), hydrous oxides of several metals dissolve with increase proton input. This causes a strong increase in the levels of toxic Al^{3+} and other metals in the soil solution (Blume et al. 2016). As a result of the decrease in pH, nitrification is strongly hampered or even completely absent in most of these highly acidified soils. This may lead to (very) high soil ammonium concentrations, with nitrate levels decreasing to almost zero (e.g. Roelofs et al. 1985). In addition, the decomposition rate of organic matter in the soil is reduced in these acidified soils, which leads to increased accumulation of litter. As a result of this cascade of changes, plant growth and species composition of the vegetation can be seriously affected: acid-resistant plant species will gradually become dominant, and several species typical to intermediate and higher soil pH will disappear. It is concluded that increased soil acidification seriously affect soil chemistry with (sometimes) lower pH, less base cations, increased toxic Al^{3+} and ammonium concentrations (e.g. Bobbink et al. 2010a, see also **Table 7**).

The effect of increased N deposition on soil acidification is substantial. On a global scale, N addition significantly reduced soil pH by 0.26 points on average for terrestrial ecosystems, causing significant decreases in soil exchangeable Ca^{2+}, Mg^{2+} and K^+, and concomitant increase in soil exchangeable Al^{3+} (Tian and Niu 2015).

Considering the strong decrease in S and moderate decrease in N deposition in Western-Europe during the last decades (Colette et al. 2016, Johnson et al. 2018, EEA 2019, Schmitz et al. 2019), a major question posed is whether soils will still suffer from acidification because the potential acidifying deposition has decreased by 50%, and is currently mainly based on N deposition. The Acid neutralizing capacity (ANC) remained unchanged in acid soils (base saturation ≤20%, pH(CaCl₂) ≤ 4.5) and decreased in better-buffered soils (base saturation >20%, pH(CaCl₂) > 4.5) (Johnson et al. 2018).

Table 7. *Effects of N on soil parameters of natural soils, their mechanisms and the ecosystem response. + denotes reported increase, -: denotes reported decrease, + / - denotes reports of both increase or decrease. Table taken from Velthof et al. (2011), adapted and expanded with more recent studies where appropriate.*

Tabel 7. *Effecten van N op bodemparameters van natuurlijke bodems, hun mechanismen en de reactie van ecosystemen. + indiceert gerapporteerde toename, -: indiceert gerapporteerde afname, + / - indiceert rapportage van zowel toename als afname. Tabel overgenomen uit Velthof et al. (2011), waar nodig aangepast en uitgebreid met recentere onderzoeken.*

Soil parameter	Mechanism	Ecosystem response	Literature
C/N ratio	Reduced variability of soil C/N ratio, due to the incorporation of surplus N in soil organic matter	Plant species richness (+ /-). Decomposition of SOM (+/-) Microbial biomass (+)	von Oheimb et al. (2008) Friedel et al. (2006) Dumortier et al. (2002) Berg (2000)
Inorganic N concentration	N deposition is close to or exceeds ecosystem N demand. Input inorganic N increases soil solution concentrations	Plant productivity (+) Leaf/needle N content (+) Litter decomposability (+) Plant species richness (-) Vascular plants wetlands (+) Microbial N immobilization (-) Nitrogen leaching (+) Soil N₂O/NO emissions (+)	de Vries et al. (2006) Corre et al. (2007) Kreutzer et al. (2009) Gundersen et al. (2006) Stevens et al. (2006)
Acidification and soil buffering capacity	Nitrification of deposited NH_3/NH_4^+ leads to H^+ formation. In the course of the acidification process base cations are leached	Available Ca and Mg (-) Al/Mn toxicity of soil pH<5.5 (+) Biodiversity (-) Microbial activity (-) Root growth (-) N-leaching (+) DOC leaching (+) Soil N₂0/NO emissions (+) Wetland CH₄-emissions (+)	Matzner and Murach (1995) Raubuch and Beese (2005) Bowman et al. (2008) Gauci et al. (2005) Evans et al. (2008)

Table 7. (continued)

Soil parameter	Mechanism	Ecosystem response	Literature
Soil C stocks and SOC stratification	Surplus N decreases fine root biomass and, thus, reduces belowground litter production, but increases aboveground plant production and litter fall.	Total soil C stocks (+) Forest floor C stocks (+) Mineral soil C stocks (+/-)	Högberg (2007) de Vries et al. (2006) Hyvönen et al. (2007) Hyvönen et al. (2008)
Soil aggregation	N can increase litterfall and improve litter quality and, thus, positively affect soil fauna and the formation of organo-mineral soil aggregates by e.g. earthworm activities	Soil aeration (+) Water infiltration (+)	Lavelle et al. (2007)
C/N/P stoichiometry	Soil N increases (see above)	In northern and central Europe and North America, increases in N deposition have reduced the C:N ratios and increased the N:P ratios of soils	Bobbink et al. (1998) Yesmin et al. (1996) Bragazza et al. (2004) Pardo et al. (2007) Sardans and Penuelas (2012) Sardans et al. (2012a) Penuelas et al. (2020)

Recently, a comparable study was conducted by de Vries et al. (2019) in which soil chemistry was repeatedly measured in 1995 and in 2015 in 16 oak forests in the Netherlands. Average base saturation of the top 30 cm of soil was between 5-40% in 1990, but in 2015 soil base saturation was in all forests less than 15%. Soils that were already highly acidic in 1995 showed minor signs of recovery, but soils with higher buffer capacity in 1995 further acidified in 2015. Both recent studies thus show that increased soil acidification still takes place in Europe in general and in the Netherlands in particular.

4.3 Aquatic ecosystems

4.3.1 Eutrophication

The total contribution of N deposition to Dutch surface waters amounted to about 26 x 10^6 kg/yr in 1990, reflecting about 12% of the total N load to Dutch surface waters (CBS et al. 2019). This amount substantially decreased down to about 12 x 10^6 kg/yr in 2016, following overall declines in N loads and thus nowadays representing about 11% of the total N loads to Dutch surface waters (CBS et al. 2019). Oligotrophic surface waters are particularly sensitive to N deposition (**Table 8**).

For instance, N deposition caused a shift from relatively balanced but predominantly N limited alpine lakes in the Rocky Mountains of Colorado (USA) toward a more consistently P limited regime (Elser et al. 2009b, Murphy et al. 2010). When lakes directly receive vast amounts of nutrients from anthropogenic activities in the surrounding watershed, the relative contribution of atmospheric N deposition to total annual N loads may not exceed 10-20% (Vitousek et al. 1997, Zhan et al. 2018). However, also highly eutrophic lakes can be at least temporarily N limited, particularly during summer when enhanced denitrification rates reduce inorganic N in surface waters (Paerl et al. 2011, Scott et al. 2019). Under these circumstances, the contribution of N deposition to total N loads may increase to up to 48%, and results in increased primary production (Zhan et al. 2018).

4.3.2 Acidification

Besides increasing bioavailable N, the acid neutralizing capacity (ANC) of aquatic systems is also impacted by N deposition, particularly of poorly buffered oligotrophic systems (**Table 8**; Lepori and Keck 2012). For instance, alpine lakes in Colorado experiencing high amounts of N deposition showed lower ANC and lower pH as compared to lakes that experienced less N deposition (Elser et al. 2009b). Similarly, many ombrotrophic fens, including those in the Netherlands, have been

significantly impacted by N deposition, through acidification and eutrophication (Hogg et al. 1995, Kooijman and Paulissen 2006, Lamers et al. 2015).

Table 8. *Overview of impacts of N deposition on some remote regions and vulnerable freshwater ecosystems, showing vulnerability to acidification and N enrichment. Table taken from Lepori and Keck (2012).*

Tabel 8. *Overzicht van de effecten van N-depositie op enkele afgelegen regio's en kwetsbare zoetwaterecosystemen, waaruit blijkt dat ze kwetsbaar zijn voor verzuring en N-verrijking. Tabel overgenomen uit Lepori en Keck (2012).*

Region	N deposition	Vulnerability to acidification	Vulnerability to nutrient-enriching effects
Adirondacks, USA	Moderate (to 12 kg N ha−1 year−1) [1]	High [2]	Likely. N limitation common in seepage lakes, but not in drainage lakes (which are already affected by N deposition) [3]
Alps, Europe	High (to ~35 kg N ha−1 year−1) on the Southern slope [4]	High on crystalline watersheds [5, 6]	Poorly known, but nutrient assays (in a watershed affected by N deposition) suggest P or no nutrient limitation [7]
Arctic	Low (0.2–0.5 kg NO3− ha−1 year−1) [8]	Extremely high, owing to negligible neutralizing capacity [8]	Likely. Shifts in diatom community compositions from the 1950's suggest synergistic N deposition and climate effects [8, 9]
Central Mountains, Japan	Moderate (to 10.5 kg N ha−1 year−1) near Tokyo [10]	Low, owing to high weathering and cation exchange rates [11]	No information found
Rocky Mountains, USA	Moderate (to 8 kg N ha−1 year−1) on the Eastern slope [12]	High, although evidence for acidification is scarce, presumably because of the moderate deposition [13]	High. N limitation common (25%) in lakes receiving low N deposition [12]
Scandes, Scandinavian Peninsula	Moderate (to 10 kg N ha−1 year−1) in the South [14]	High, especially in Norway [15]	High. N limitation common (75%) in lakes receiving low N deposition [14]
Sierra Nevada, USA	High (to ~35 kg N ha−1 year−1) around greater Los Angeles [16]	High, owing to weathering-resistant geology [17]	Likely. Early studies in lake Tahoe (before it was affected by nutrient enrichment) suggest primary N limitation [18]
Šumava Mountains, Central Europe	High (to ~35 kg N ha−1 year−1) [19]	High, owing to bedrock of granite and gneiss [19]	No information found
Tatra Mountains, Central Europe	High (wet-only deposition to 20 kg N ha−1 year−1) [19]	High, with evidence of freshwater acidification [19]	No information found

References: [1] = Ollinger et al. (1993); [2] = Driscoll et al. (2003); [3] = Saunders et al. (2000); [4] = Rogora et al. (2001); [5] = Marchetto et al. (1994); [6] = Lepori et al. (2003); [7] = Robinson et al. (2003); [8] = Wolfe et al. (2006); [9] = Holmgren et al. (2010); [10] = Mitchell et al. (1997); [11] = Ohte et al. (2001); [12] = Elser et al. (2009a) [13] = Vertucci and Corn (1996); [14] = Bergström et al. (2008); [15] = Henriksen et al. (1992); [16] = Fenn and Poth (1999); [17] = Stoddard (1995); [18] = Goldman (1988); [19] = Kopáček et al. (1995).

4.4 Summary conclusions

Nitrogen eutrophication from the air is a major problem in many natural and semi-natural, terrestrial ecosystems, leading to increased N cycling. N deposition will cause N enrichment of naturally oligotrophic ecosystems in both the terrestrial and aquatic biomes, including grasslands, shrublands, forests, streams, fens and mountain lakes. Moreover, in aquatic systems, enrichment effects may also have significant ecological implications in eutrophic waters when nitrogen is temporally limiting, which is likely to occur in summer when nitrification rates are high.

The N availability in soil and water gradually increases with higher N loading from the atmosphere and in the longer term soil C:N ratio will considerably decrease. At the same time, soil and water N:P ratio has consistently been reported to increase. These changes in soil and water chemistry and relative abundances of elements may have severe consequences for the chemistry of the vegetation, and thus on higher trophic levels, too (see chapters 2-6).

Soil acidification is also an important effect of atmospheric N deposition. It leads to loss of ANC, a lower soil pH, increased leaching of base cations (Ca^{2+}, K^+, Mg^{2+}) and increased concentrations of potentially toxic metals (e.g. Al^{3+}). In addition, it causes a decrease in nitrification rate – thus increasing the concentration of ammonium relative to nitrate, and in litter accumulation, due to the reduced decomposition rate. The consequences of severe soil acidification can also significantly affect plant chemistry, potentially impacting higher trophic levels (see chapters 2 and 5).

5 Effects of N deposition on producer quality and consumer performance

Primary producers directly rely on the abiotic environment for their resources such as light and nutrients. Consequently, they depend on specific abiotic conditions such as the limiting resource, and shifts in environmental conditions in these resources can alter their elemental and biochemical composition (Geider and La Roche 2002, Sterner and Elser 2002, Elser et al. 2010a). An increase of N deposition leads to accumulation of N compounds in the soil, water or sediment, resulting in higher N availabilities and changes of producer species interactions. This ultimately leads to changes in N cycling, species composition and producer species diversity (e.g. Bobbink et al. 1998, Schindler 2006). This eutrophying effect chain can be highly influenced by other factors, such as other global change factors including rising temperature, CO_2 and precipitation changes (Feuchtmayr et al. 2009, Sinha et al. 2017, Welti et al. 2020), as well as limitation by other resources (P, K, Mg or water) (Aerts and Bobbink 1999). The concentration and composition of N compounds, both protein N and non-protein N, can be highly influenced during this N enrichment process.

N deposition may furthermore lead to loss of the acid neutralizing capacity (ANC) and increased soil or water acidity that in turn may first increase the bioavailability of base cations (Na, K, Mg, Ca) in soil and poorly buffered surface waters such as fens, and in more advanced stages of soil acidification, in leaching of base cations and in increasing the bioavailability of metals (Al, Fe). Soil acidification hence influences root uptake of cations considerably. As many cations are crucial for cellular functioning, producers eventually experience detrimental effects of acidification due to (micro-)element limitation (leaching) and/or metal toxicity (increased Fe and Al) (eg. Tian and Niu 2015, Blume et al. 2016).

In this chapter, we first describe the mechanisms how increased N deposition alters contents of macro- and micro-elements and of nutrients in primary producers, in reviewing existing literature that focus on the effects of N-deposition mediated shifts in the nutritional quality of producers. We follow the same structure of increasing complexity as used in chapter 0 in our definition of nutritional quality. For each of these nutritional aspects, we then review studies that specifically focus on the effects of N-deposition mediated shifts on the response of consumers, and finalize for each nutritional aspect with a summary conclusion paragraph in which we integrate the findings with those formulated under chapter 0.

5.1 Effect of N deposition on soil- and water-plant interactions

5.1.1 Differential effects of reduced or oxidized N in producer response

In many European regions with increased N deposition, a high proportion of the deposited N originates from ammonia (e.g. Asman et al. 1998, Fowler 2002). This may cause a change in the dominant N form in the soil from nitrate to ammonium, especially in habitats with low nitrification rates (pH < 4.5) or lead to an increased ammonium uptake by the above-ground plant parts. The response of sensitive plant species can be significantly affected by this change. Species adapted to calcareous or acidic soils are able to use nitrate or a combination of nitrate and ammonium as the inorganic N source, whereas early studies showed that species of acid habitats generally use ammonium (Gigon and Rorison 1972, Kinzel 1982, for overview see Lambers et al. 2008). Laboratory and field studies demonstrate that the performance and persistence of most understory species of deciduous forests in southern Sweden improves when not only ammonium but also

nitrate can be taken up (Falkengren-Grerup et al. 1998, Olsson and Falkengren-Grerup 2000). One of the impacts of increased ammonium uptake is the reduced uptake of base cations and the exchange of these cations (K, Ca & Mg) to the phyllo- or rhizosphere. Ultimately this can lead to severe nutritional imbalances, especially N/K or N/Mg, which are considered to be important in the decline in tree growth in areas with high ammonia/ammonium deposition (Nihlgård 1985, van Dijk et al. 1990).

High ammonium concentrations in soil or water are also toxic to sensitive producer species, causing disturbed cell physiology, internal cell acidification, accumulation of N-rich amino acids, poor root development, and finally, inhibition of shoot growth. Strong evidence exists that several endangered vascular plants, bryophytes and lichens of habitats of intermediate pHs (between 4.5 to 6.5) such as grasslands, heathlands, fens and several deciduous forest types are (very) intolerant to increased ammonium concentrations and/or to high NH_4/NO_3-ratios (De Graaf et al. 1998, Lucassen et al. 2003, Paulissen et al. 2004, van den Berg et al. 2005, Kleijn et al. 2008, van den Berg et al. 2008, De Graaf et al. 2009, Verhoeven et al. 2011, Paulissen et al. 2016). For additional information on the differential responses to reduced and oxidized N deposition, see the reviews of Britto and Kronzucker (2002) and Stevens et al. (2011).

Ammonium toxicity is also clearly demonstrated for macrophyte communities of weakly buffered soft-water lakes: increased high NH_4/NO_3-ratios clearly inhibited the growth of the typical macrophytes of this system, but stimulated the growth of some resistant species (*Juncus bulbosus* and *Sphagnum cuspidatum*), leading to strong monocultures of species (Roelofs 1983, Schuurkes et al. 1986, Smolders et al. 2002). Furthermore, ammonium toxicity has been shown for submerged macrophytes, such as *Stratiotes stratioides* and the seagrass *Zostera marina* (Smolders et al. 1996, van Katwijk et al. 1997). In well-buffered aquatic ecosystems, NH_4 is rapidly taken up and assimilated by phytoplankton, or converted to NO_3 by nitrifying bacteria, and N availability these aquatic systems is mostly represented by enhanced concentrations of NO_3. In these systems, the NH_4/NO_3 ratio will thus continue to be low until the acidifying effect of this increased nitrification results in severe acidification, which in turn suppresses nitrification rates with lowered NH_4 conversion and subsequent build-up of NH_4. Although NO_3 can be taken up by aquatic plants in the littoral zone, or denitrified by bacteria and thus be lost to the atmosphere through N_2, higher N deposition generally leads to enhanced phytoplankton biomass and shifts from N to P limitation in such well-buffered aquatic habitats (Bergström and Jansson 2006, Elser et al. 2009a, Hessen 2013, Meunier et al. 2016). These shifts in relative N availabilities may also have consequences for the phytoplankton community composition, for example with reduced abundance of N_2 fixing cyanobacteria and lower biodiversity due to imbalanced resource supplies (Elser et al. 2009b, Liess et al. 2009, Hessen 2013).

It is clear that producer species and habitat types differ strongly in their responses to high soil and water NH_4 concentrations. This may also results in different impacts with respect to N deposition in total uptake of N by producers, generally resulting in disrupted (i.e. unregulated) uptake of N by NH_4 sensitive species. This hence increases tissue N content beyond beneficial concentrations, and in relatively low increases in plant tissue N concentration in ammonium-tolerant species, mostly the superior competitors. However, only an insufficient number of studies exist that performed N-addition experiments with differential treatments of reduced and oxidized N to give general conclusions on the changes in N form.

5.1.2   Effects of increased N deposition on the amino acid chemistry of producers

One of the well-known changes in plant chemistry in regions with high N deposition is the increase in the concentrations of N-rich free amino acids, especially arginine, asparagine and glutamine, and thus in the general composition of amino acids of producers. This mechanism helps to reduce direct NH_4 toxicity to the plant (Britto and Kronzucker 2002, Stevens et al. 2011). Furthermore, the increase of the N-rich free amino acid concentration has often be considered as one of the best early-sign indicators for increased N deposition in an originally low N deposition background area (Løkke et al. 1999).

This plant response to increased N inputs with considerable higher levels of N-rich free amino acids have been found in several habitats, such as bogs (Tomassen et al. 2003, Fritz et al. 2012), fens (Paulissen et al. 2016), heaths (Power and Collins 2010), shallow waters (Smolders et al. 1996) and in coniferous forests (Huhn and Schulz 1996, Løkke et al. 1999). The increase in N rich amino acids have been reported for many plant groups, including bryophytes (Paulissen et al. 2016, Xu et al. 2018), coniferous trees (Huhn and Schulz 1996) and dwarf-shrub species (Power and Collins 2010). The outcome of several studies indicates that this effect on N-rich free amino acids is more common if ammonium is the dominant N form and present in increased concentrations in the soil or water.

5.1.3 N-deposition mediated shifts in mycorrhizal communities

Both review articles of Lilleskov et al. (2019) and Stevens et al. (2011) confirm the clear and strong sensitivity of mycorrhizal fungal communities to N inputs, and community functional shifts may be contingent on P availability (Lilleskov et al. 2019). Combined with possible decreased P-availability caused by soil acidification (Walker and Syers 1976), this may affect the P-content of the vegetation, leading to even stronger shifts in plant C/N/P-ratios (Vogels et al. 2017). However, soil type, pH and redox status all greatly modify the degree and severity of such mycorrhizal mediated shift in macronutrient supply. The effect of soil mycorrhizal interactions under increased N deposition is therefore highly dependent on the specific habitat conditions of the ecosystem under investigation.

5.2 N deposition effects on C:N:P stoichiometry

5.2.1 Effects on producer quality

Nitrogen is the limiting nutrient for plant growth in many European natural and semi-natural terrestrial ecosystems, especially under oligotrophic and mesotrophic conditions. Increased N deposition results in the short term in an increase in the availability of inorganic N in the topsoil. This gradually leads to an increase in plant productivity in N-limited vegetation, and thus to higher annual litter production and litter with high concentrations of N. Because of this, N mineralisation will also gradually increase, which, in turn, may increase plant productivity. This is a positive feedback, because higher N mineralisation leads to higher N uptake and its subsequent effects (Bobbink and Hettelingh 2011). The rate of N cycling in the ecosystem is clearly increased in such situations, although the response time to increased N inputs can be long in highly organic soils (with high C:N ratios), or, indeed in any soil with large potential N sinks.

When N is no longer limiting in the ecosystem, plant growth becomes limited by other resources, such as P, K, Mg, or water (Aerts and Bobbink 1999). In this situation, the productivity of the vegetation will not increase any further with continuing N inputs. However, primary producers N concentrations substantially increase when N availability continues to increase. This results in (much) higher concentrations of N-based compounds in primary producers, that may significantly affect the palatability for herbivores or the sensitivity to pathogens. In conclusion, increased N availability results in significant shifts towards lower C:N and higher N:P ratios in primary producers in general (Sardans et al. 2012a; **Figure 17**).

In aquatic systems, numerous studies have tested the impact of limitation by N on elemental ratios, and demonstrated generally increased C:N and decreased N:P ratios in primary producers (Geider and La Roche 2002, Hillebrand et al. 2013). Enrichment effects by N on phytoplankton stoichiometry are less often studied, though when exposed to N enrichment, natural phytoplankton communities showed an increased growth in a large number of systems, thus suggesting N limitation in these systems (Elser et al. 2007). It is conceivable that under such conditions, N enrichment will ultimately lead to decreased C:N and increased N:P ratios. Indeed, high N deposition lakes have been shown to generally exhibit higher phytoplankton N:P ratios as compared to low N deposition lakes (Elser et al. 2009a). Moreover, phytoplankton stoichiometry may also alter through shifts in phytoplankton species composition, as phytoplankton classes were shown to have different elemental ratios (Hillebrand et al. 2013, Garcia et al. 2018).

Impacts of N deposition on producer stoichiometry will largely depend on prevailing N concentrations. When N is limiting, N deposition will often lead to enhanced growth and increased N:P ratios since N limitation is alleviated and cellular N:P balance is restored to optimal conditions (Elser et al. 2009b).

The impacts of N enrichment on non-limiting phytoplankton are less clear and poorly studied. When N is in excess, additional N is unlikely to cause changes in N:P ratios since uptake and assimilation of N is already saturated. Yet, when N concentrations are non-limiting but low, additional N may possibly be taken up and accumulated in phytoplankton as storage, thus raising N:P ratios. Moreover, increased N deposition can exhibit seasonal effects on ecosystem functioning as exemplified by Chen et al. (2018). In their study system (lake Taihu, China), N deposition contributed to over 12% of total N inputs. This relatively minor contribution had a large impact on growth and possibly stoichiometry of cyanobacteria blooms that were shown to become N limited during summer season, when high denitrification rates cause substantial net losses of N; hence enforcing N-limitation in this season (Paerl et al. 2015, Scott et al. 2019).
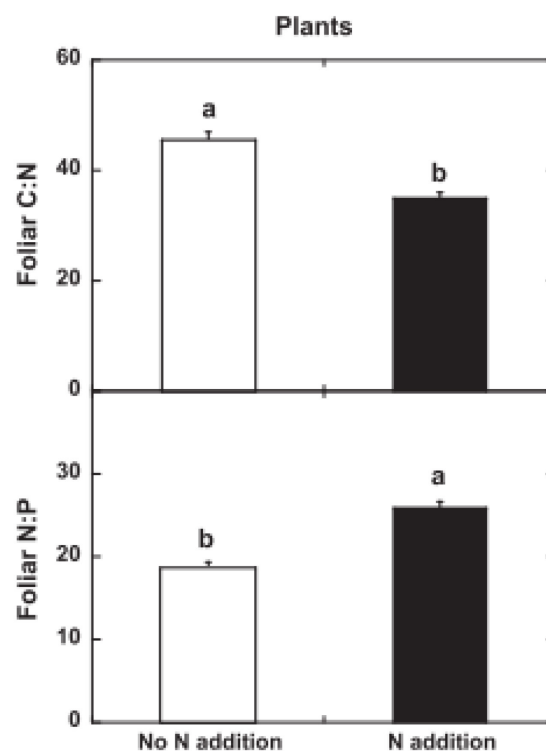
Figure 17. *Metadata-analysis of 31 different experimental results in the case of leaf molar C:N ratio and of 22 different experimental results in the case of leaf molar N:P ratio growing at ambient and in N addition treatment. Different letters indicate statistically different values (P < 0.05). Figure and analysis from Sardans et al. (2012a).*

Figuur 17. *Metadata-analyse van 31 verschillende experimentele resultaten van blad molaire C:N-verhouding en van 22 verschillende experimentele resultaten van blad molaire N:P-verhouding onder omgevings- versus N-additie behandeling. Verschillende letters geven statistisch verschillende waarden (P <0.05). Figuur en analyse uit Sardans et al. (2012a).*

5.2.2 Consumer response to producer C:N:P stoichiometry shifts

Reports of consumer responses to shifts in producer C:N:P vary greatly in their outcome. Producer decreases in C:N ratio has often been shown to increase consumer fitness (White 1993, Kurze et al. 2017), but opposite consumer responses to N enrichment are also reported (Fischer and Fiedler 2000, Kurze et al. 2018). These different outcomes of (experimental) N enrichment studies on consumer performance may originate from contrasting life-history strategies of the consumer species under study (see pars. 2.2.2.6, 0, 2.4.3.3, 2.6.2 and 2.7), from induced shifts from N (i.e.

low C:N is beneficial) to P (i.e. low N:P is beneficial) limitation in consumers or a combination of both. Also, changes in producer allelochemical concentration may greatly modify the response of consumers to increased N supply to the producer (see pars. 2.6 and 5.6). In nutrient poor, acidic heathland ecosystems in the Netherlands, increases in producer N:P stoichiometry have been shown to significantly impact invertebrate herbivore density and species richness; which could be linked to soil nutrient and buffer status (Vogels et al. 2017). Soil acidity and a concomitant decrease in P- availability in producers significantly increased producer tissue N:P ratio, which was further exacerbated by a concomitant removal of plant available P in resulting from intensive management schemes aimed at maximizing N removal. This increased producer N:P ratio has also been found to negatively affect fitness of the field cricket *(Gryllus campestris),* a characteristic and endangered species of heathland ecosystems (Vogels et al. 2011, Vogels et al. 2016). Management induced changes in soil N to P balances have also been found to explain loss in herbaceous plant species abundance and richness in these systems (Vogels et al. 2020), which may further hamper consumer fitness through loss of complementary food items (see par. 2.4.3).

In contrast, a N deposition induced increase of *Calluna* tissue N (and hence decreased C:N ratio) has been linked to increased performance (Van der Eerden et al. 1991) and subsequent outbreak events of Heather beetle (*Lochmaea suturalis*) (Brunsting and Heil 1985, Taboada et al. 2016), effectively resulting in massive heather die-back and stimulating grass-encroachment of heathlands (Brunsting 1983, Berdowski 1987, Berdowski 1993). Winter moth (*Operophtera brumata*) caterpillars (a generalist species feeding on woody plants) also showed increased larval development, growth rate and pupal development in N-treated *Calluna vulgaris* (Kerslake et al. 1998). In this study, phenolic content did not change significantly in N treated plants, while C:N ratio did change significantly suggesting a single role of lowered C:N ratio in promoting caterpillar growth. Kurze et al. (2018) however found for several *Rumex* and grass feeding Lepidoptera species a reduced caterpillar survival rate under increased host plant N supply, although most N supply levels were much higher (90-300 Kg^{-1}ha^{-1}y^{-1}) than relevant with respect to N deposition. For one species (the Sooty copper, *Lycaena tityrus*), reduced survival however already became apparent under the N deposition relevant supply rate of 30 Kg^{-1}ha^{-1}y^{-1}, and host plant tissue C:N ratio was not significantly different between N addition treatments. In a greenhouse experiment with the Small copper (*Lycaena phlaeas)*, pupal weight was found to correlate positively with *Rumex acetosa* N content, but larval growth was significantly reduced under treatments that resulted in lower plant tissue P content (Weijters et al. 2018), suggesting a role of both N and P in controlling larval development.

Hendriks et al. (2013) found that in the Netherlands, only those orthopteran species with low dispersal capacity were negatively affected by nitrogen deposition, suggesting a role of life history strategy for changed food quality in determining fitness effects (see paragraph references made earlier). However, other environmental factors such as vegetation height, soil conditions and soil temperature were more important in explaining species distribution trends in Orthoptera. The relatively low sensitivity for N deposition in Orthoptera might be explained by the relative high tissue N content, and thus higher N demands for this species group (Fagan et al. 2002, Woods et al. 2004). Tao and Hunter (2012) also concluded that increased N deposition does not inevitably leads to increased P limitation in specialist herbivores, and that species-specific nutrient demands as well as plant defense responses to increased nutrient availability determine species performance to increased N deposition.

For aquatic consumers, numerous examples have shown how limitation by P causes an increase in producer C:P ratios and declines in consumer performance such as growth and development rates (for a review see Hessen et al. 2013). Besides, excess P has also been shown to come at a cost to consumers, indicating optimal C:P ratios (Boersma and Elser 2006, Zhou and Declerck 2019). The few studies investigating the effects of producer N limitation on aquatic consumer performance are in line with those reported for the effects of P limitation, and seem also to lead to declines in performance as have been observed for tadpoles (Stephens et al. 2017), rotifers (Golz et al. 2015), and copepods (Van Nieuwerburgh et al. 2004). N deposition could thus possibly enhance consumer

performance, though this may also be temporarily as under chronic or high N deposition, it can cause shifts in limitation from N to (co-limitation with) P, thereby limiting or reducing consumer growth and development (Elser et al. 2009a, Bergström et al. 2018).

The way consumers respond to shifts in producer stoichiometry depends on their homeostatic regulation. Stoichiometric homeostasis may optimize growth of consumers, as optimal conditions for cellular processes are maintained. At the same time, however, a flexible stoichiometry may also be beneficial, for example when it allows storage of elements (Meunier et al. 2014). While the negative effects of stochiometric imbalances are generally evident for first order consumers, it seems that they may trophically upgrade the low-quality producer stoichiometry for higher order consumers (Malzahn et al. 2010, Golz et al. 2015). The extent of stoichiometric homeostasis differs between consumers (Meunier et al. 2014, Golz et al. 2015), and the impacts of N deposition on consumers will thus depend not only on stoichiometric variation of the producers, but also to the stoichiometric homeostasis of the consumers.

Nitrogen deposition induced changes in producer C:N:P stoichiometry thus depend on the degree of changes in producer C:N:P ratio. Subsequently, the direction of consumer response depends on whether this results in a shift towards the consumer specific TER or away from it, which in turn depends on the specific consumer physiology and/or life history tactic. In general, such changes in producer C:N:P ratio could however result in an overall decrease in consumer community species richness (Lee et al. 2017), as it promotes outbreak events of a small subset of species, and concomitant declines in other species, depending on the species specific TER for C:N:P.

5.3 N deposition effects on other elements and trace metals

5.3.1 Effects on producer quality

Increased N deposition from the atmosphere may change the nutritional value of terrestrial producers by increasing the N concentration in the foliage and/or decreasing the (relative) uptake of other macronutrients (for the impacts upon P and the N/P ratio, see previous section). Depending on the soil type, both K and/or Mg concentrations are lowered. Thus, the ratios between N on the one hand and K and Mg (sometimes also for Ca) on the other hand, tend to increase considerably. These changes were found in both field observations across gradients in N deposition and in experimental N additions (**Figure 18**; Flückiger and Braun 2011, Lucassen et al. 2014, Jonard et al. 2015).

A decrease in K and Mg concentrations and much higher N to base cations ratios in plant leaves or needles may be caused by (a combination of) (1) reduced uptake as a result of Al toxicity to roots after soil acidification (Godbold 1991, Rout et al. 2001), (2) as a result of competition with ammonium in the root uptake system (Gerendas et al. 1997), (3) as a result of N impacts on (ecto)mycorrhizal fungi (Yesmin et al. 1996, de Witte et al. 2017), (4) increased leaching of these cations during canopy uptake of ammonia/ammonium (Roelofs et al. 1985, Bobbink et al. 1992) or (5) by a decreased supply rate of nutrients other than N due to increased leaching of these macronutrients (base cations: K, Mg and Ca) caused by increased soil acidification (see par 4.2.2).

The nutritional imbalance between N and base cations (especially Mg and K) caused by increased N deposition has especially been demonstrated in many studies of coniferous and deciduous forests. For a detailed review on this nutritional imbalance, see chapter 8 in Bobbink and Hettelingh (2011). Furthermore, this reduction of the concentration of base cations was also found in N studies with heathland and fen (bryophytes), and especially related to high ammonium availability (De Graaf et al. 1998, Lucassen et al. 2003, van den Berg et al. 2005, Paulissen et al. 2016). In some of the last mentioned studies, leaf Mn concentrations were reduced by high ammonium levels, too. To conclude, reduced concentrations of base cations – and significantly increased N to base cations ratios – are commonly observed with high N deposition in European forests, but also demonstrated for other ecosystem types.
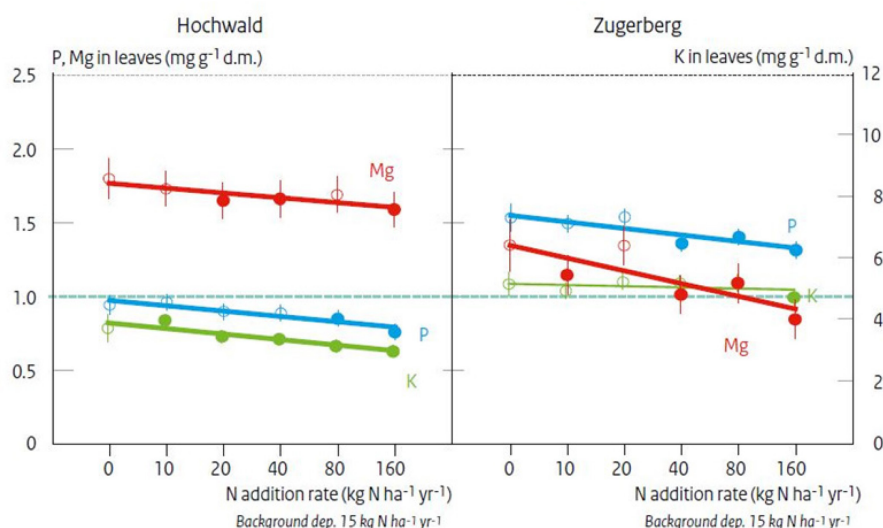
Figure 18. Nutrient concentrations in the foliage of beech subjected to different N treatments on calcareous soil (Hochwald) and acidic soil (Zugerberg) (averages of 8 samples over an experimental period of 14 years). Bars: 95% confidence intervals. Bold lines: Regression significant at p<0.05, filled points: difference to control significant. Dashed color line: lower limit for optimum nutrient concentrations, according to Stefan et al. (1997). Modified from Flückiger and Braun (2011).

Figuur 18. Nutriëntenconcentraties in beukenblad onder verschillende N-behandelingen op kalkrijke (Hochwald) en zure bodem (Zugerberg) (gemiddeld 8 monsters over een experimentele periode van 14 jaar). Bars: 95% betrouwbaarheidsintervallen. Vetgedrukte lijnen: Regressie significant bij p <0,05, gevulde punten: significant verschil. Onderbroken kleurlijn: ondergrens voor optimale voedingsconcentraties, volgens Stefan et al. (1997). Aangepast naar Flückiger en Braun (2011).

Quantified effects of increased N deposition on trace elements in either terrestrial or aquatic ecosystems are extremely scarce. Several interactions however exist between N metabolism and various trace elements, e.g. N_2 fixating nitrogenase enzymes (Fe, Mo, V), nitrate reductase (Mo), hydrolysis of urea (Ni), amino acids (Mn) and peptides. Changes in N availabilities may thus strongly alter trace element demands of producers. For instance, enhanced N availability may lead to down-regulation of N_2 fixation in marine cyaonobacteria, thereby possible reducing cellular Fe and Mo contents (Tuit et al. 2004). Moreover, enhanced N loading of coastal waters may lead to Si limitation in diatoms that require Si for their cell walls. Consequently, enrichment by N can cause shifts in phytoplankton community composition, with less diatoms and more non-siliceous phytoplankton (Garnier et al. 2010), which will presumably lead to a reduced Si content in the producer community.

In high nutrient low chlorophyll-a (HNLC) oceanic regions, Fe is a main limiting nutrient for primary producers (Moore et al. 2013). The majority of Fe in one of these regions was shown to attributed to the smallest group of phytoplankton (picophytoplankton; 0.2-2 um), which also showed highest Fe:C ratios (McKay et al. 2005). In freshwater lakes, trace metal contents of phytoplankton (Hg, Pb, Cd, Cu, Mn, Co, Ni and Cr) and the trace metal to P ratio were inversely correlated to the trophic state of the lake, and it has been suggested that trace metal contents decrease due to growth dilution (Gormley-Gallagher et al. 2016). Thus, under high N and P conditions, trace element contents in phytoplankton are likely to decrease, either because they become limiting, or because of growth dilution.

Increased N-deposition can also alter (trace) metal availability and (trace) metal content in producers, either directly via disruption of metal homeostasis under NH_4-toxicity (Mehne-Jakobs and Gulpen 1997, Britto and Kronzucker 2002) or indirectly via increased leaching of trace metals and concomitant increase of toxic heavy metals resulting from soil acidification (Haynes and Swift 1985, Gjengedal 1996, Sparling and Lowe 1998, van Oene 1998, Gjengedal et al. 2015). The

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elemental content of producers is however highly dependent on other factors such as mineralogy of the soil substrate (Gjengedal et al. 2015), phylogeny (Heilmeier et al. 2000), soil moisture (Borer et al. 2019) and temperature (Tian et al. 2019). Hence, generalizations of the impact of N deposition on the composition of (trace) elements in producers (trace) elemental composition cannot be made and should instead be investigated within the context of specific ecosystems. In general, the nature and degree of the limiting element (N vs. C or P), the soil or water buffer status (highly buffered vs acidic or acid environment) and (trace) metal availability will determine the sensitivity of an ecosystem to experience changed (trace) metal content in producers under increased N deposition.

5.3.2 Consumer response to changed producer elemental composition

5.3.2.1 Metal deficiency

Of all ions, Ca is the most often reported nutrient to be deficient in consumers as a result of soil acidification and nitrate-driven leaching (Perakis et al. 2013). Soil acidity itself is not a good predictor of Ca availability for herbivores (Levia et al. 2015). The Ca flux in litter substrate can be substantial, even in acidic soils. Ca is not retracted by plants before leaves are shed, so in temperate forests, much of the Ca accumulated by trees is deposited yearly on the forest floor (Chen et al. 2013). This probably enabled large litter- dwelling detritivorous arthropods with Ca-bearing exoskeletons such as millipedes and isopods to colonize detritus rich ecosystems, even in nutrient- poor, acidic environments. Nevertheless, soil acidity does restrict many species belonging to this group to live in the soil substrate as Ca from their exoskeletons would dissolve at low pH. The most persistent species have evolved mechanisms to reduce exoskeletal damage by low pH, for instance by developing a thick cuticle (for example in *Porcellio scaber*), but in highly acidic soils also these species cannot maintain themselves in the soil litter layer. Some snail species, for example *Discus rotundatus*, and *Clausilia bidentata*, circumvent this problem by living in or on tree stumps or trees (cf. Kappes and Topp 2014). Under increasing soil acidification, and associated decreasing Ca availability and increasing Al availability, the abundance of these high acidity-adapted species is also strongly reduced, and ultimately leads to the local extinction of these species.

Invertebrates which fortify their exoskeletons with Ca obviously make good sources of Ca for vertebrate predators (Graveland 1995, Pabian and Brittingham 2007). The absence or low availability of such prey types is the most important contributing factor in overt Ca deficiency symptoms in vertebrates (Bures and Weidinger 2003, Dawson and Bidwell 2005, Gosler and Wilkin 2017). Many bird species supplement their diet with Ca- rich prey types, but also parts of snail shells or other Ca- rich substrates. Even at sites without apparent Ca deficiencies in birds, lime application (dolomitic limestone sand, 4.5 ton/ha; Pabian and Brittingham 2007) proved to increase snail abundance and populations of various bird species (Pabian and Brittingham 2011, Pabian et al. 2012a) Millipedes did not respond to increased Ca availability. In birds, apart from the density effect, clutch size increased significantly. Concurrent effects included increased soil pH, Ca and Mg levels, increased plant growth, higher forb Ca and Mg levels, and lowered soil Al and Mn concentrations (Pabian et al. 2012a, Pabian et al. 2012b). Ca deficiencies are unknown from the highest orders of carnivores, as their vertebrate prey already accumulated sufficient amounts of Ca, preventing shortages in top-predators.

In young birds and embryos, deficiencies in elemental nutrition other than Ca give rise to growth anomalies such as maldevelopment of the spinal cord, muscle spasms, and perosis (slipped tendon) (Leeson and Summers 2001, Van den Burg 2017a). Such anomalies appear extremely rare, also in forests where due to acidification, Ca shortages in birds are severe (for embryos: van den Burg 2009; for chicks: pers. obs. A. van den Burg). The most parsimonious explanation for this absence may be the much higher demand for Ca in vertebrate animals compared to other elements, resulting in earlier manifestations of Ca-deficiencies compared to other elements. In insects, low elemental leaf concentrations could also not explain the variance in herbivory levels between oak stands (van den Burg et al. 2014).

In aquatic (marine) systems, trace elements often (co-)limiting include Fe and Zn, especially in the areas with high nutrients but low chlorophyll-a concentrations (i.e. HNLC areas; Moore et al. 2013). In consumers from one of such areas the Fe:C and Zn:C ratios showed a respective decrease and increase with consumer size (Baines et al. 2016), indicating that Fe demands are higher in smaller zooplankton, while Zn demands are higher in larger zooplankton. The Fe:C and Zn:C ratios tended to be higher for zooplankton as compared to one of the phytoplankton groups (i.e. flagellates), yet both ratios fell within the range of another group (i.e. diatoms). As the latter is only available in relatively low densities, zooplankton may only be able to fulfil their trace element demands if they are able to selectively forage (Baines et al. 2016).

At this moment, evidence that shortage or excess of trace metals in producers (induced by nitrogen enrichment or soil acidification), has cascading effects on primary consumers and higher trophic levels is lacking (Baines et al. 2016, Nijssen et al. 2017).

5.3.2.2 *Metal toxicity*

Acidification can result in the exposure of animals to toxic elements, especially heavy metals, such as Al, Cd, Hg, and Pb, ultimately affecting species fitness (Leuven et al. 1986, Schreiber and Newman 1988, Scheuhammer 1991, 1996). High levels of Cd have also been reported in red deer *Cervus elaphus* and wild boar *Sus scrofa* from the Veluwe (Wolkers 1994) and may impact amphibians in acidified pools in the Netherlands additional to the effect of low pH (Leuven et al. 1986). A concomitant decline in non-toxic (essential) metals such as Ca further exacerbates heavy metal toxicity, through increased uptake of heavy metals under low Ca conditions (Scheuhammer 1996). Some of this exposure is direct (i.e. not through food quality) such as fish gills exposed to excessive Al, which can have a great negative impact on freshwater fish populations (Rosseland et al. 1990).

Through fish and freshwater invertebrates, food quality of other animal species may be reduced (Schreiber and Newman 1988). Comparative studies of heavy metal concentrations in wildlife heavy metal concentrations from acidified and unaffected sites show that these toxic compounds do bioaccumulate as a result of their higher availability in acidified soils (Crichton and Paquet 2000). However, although concentrations in organs such as the liver and kidneys may exceed recommended levels for human consumption in ungulates, no noticeable impacts of heavy metal accumulation on animal populations have been reported (studied in ungulates, large terrestrial and aquatic carnivores, birds, fish, aquatic invertebrates; Schreiber and Newman 1988, Crichton and Paquet 2000). Increased heavy metal concentrations can however attribute to a loss of Ca rich prey items for vertebrate predators resulting in significant fitness decrease as shown for pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient (Belskii and Grebennikov 2014). Whether this also occurs under non-polluted conditions (i.e. only resulting from acidification induced increases of the bioavailability of ambient soil heavy metals) has not been investigated and thus remains unproven.

5.4 N deposition effects on proteins, carbohydrates and fatty acids

5.4.1 Effects on producer quality

Production of nitrogen containing biochemicals, such as proteins, directly depends on the availability of nitrogen, and generally decreases when nitrogen becomes limiting or, vice versa, increases with nitrogen addition when limitation is alleviated (Geider and La Roche 2002, Sterner and Elser 2002, Gao et al. 2018). In contrast, C containing compounds such as carbohydrates and fatty acids were shown to generally increase when C is in relative excess, for example under N or P limiting conditions (Geider and La Roche 2002, Sterner and Elser 2002, Gao et al. 2018). Some phytoplankton species, including cyanobacteria, may accumulate nitrogen into cyanophycin, which is a polymer of arginine and aspartic acid and used as storage molecule, and particularly synthesized upon a nitrogen pulse to quench intracellular ammonium toxicity (Allen and Hawley 1983, Van de Waal et al. 2010). As such, total N levels in producers may increase while compounds

such as complex proteins do not. Thus, if N deposition alleviates N limitation in phytoplankton, it may cause an increase in their protein contents (but this may not be true for complex proteins), but a decrease in fatty acid an lipid levels, though these latter effects will also depend on the availabilities of other nutrients.

5.4.2   Response of consumers to changed protein concentration

As indicated in the previous paragraph, often an increase in total protein content of producers has been reported as a result of increased N deposition. However, trees, heather and grasses growing under conditions of high soil acidification and resulting high shortage of mineral nutrients can also exhibit lowered protein content, despite exhibiting increased free amino acid levels and total tissue N (Vogels et al. 2011, van den Burg et al. 2014). Under these conditions, plants apparently cannot synthesize all amino acids at the required ratios for optimal protein synthesis. As a result, the quality of leaf matter for herbivores in terms of protein content is reduced (Vogels et al. 2011, van den Burg et al. 2014). In field ecological studies however, C:N ratio in producers is often used as a proxy for protein content. This is however not always of particular value in producer-consumer interactions as it applicability is contingent on other soil properties such as soil P and K availability and soil buffer status (and see remarks in paragraph 5.5). No N deposition specific studies have been found that focus on carbohydrate:protein effects specifically. As most studies use producer C:N ratio as a proxy for carbohydrate:protein ratio, we refer to paragraph 5.2 for general nitrogen deposition mediated effects on carbohydrate:protein ratios. As the C:N ratio in producers does not necessarily reflect protein:carbohydrate ratios (as discussed earlier in this paragraph), we also conclude that there exists a knowledge gap in N deposition induced changes in protein:carbohydrate ratios and the implications of these changes when applied to consumers under the framework of nutritional geometry.

5.5 N deposition effects on micronutrients

5.5.1   N-deposition driven micronutrient changes in producers

Within the context of N deposition, the root causes of micronutrient deficiencies originate from either acidifying or eutrophicating effects of soil and water by nitrogenous compounds. In the case of soil nutrient limitation, the effects can be quite direct, as soil acidification may lower nutrient availability to plants, being the fundamental cause of deficiency symptoms throughout the food web (e.g. calcium, cobalt). Unravelling other secondary routes however, such as the effect of increased nitrogen availability on plant tissue amino acid composition and vitamin B2 deficiencies in animals therefrom, relies much stronger on physiological studies in plants and animals and multi-trophic interactions. This complicates micronutrient studies and often makes them highly interdisciplinary, which constitutes underrepresentation of micronutrient studies in literature.

There is some evidence of the impact of nitrogen deposition on thiamine deficiency. Thiamin deficiency may occur in eutrophic waters with algal blooms, which are associated to a number of food quality issues, similar to fatty acid deficiencies, and toxic effects of NPN. Harmful algal blooms lead to thiamine deficiencies, which are enhanced by increased nutrient input (such as nitrogen), increased turbidity, over-fishing and low meso-zooplankton biomass (Ejsmond et al. 2019). Moreover, in eutrophic productive coastal waters, phytoplankton was shown to become limited by thiamine as well as vitamin B12 as result of uptake by heterotrophic bacteria, which compete for the same micronutrients (Koch et al. 2012). In terrestrial ecosystems, only few species produce thiaminases (e.g. some ferns in the Australian outback; Kraft and Angert 2017), and therefore N deposition induced thiamine deficiencies in terrestrial habitats are rare.

5.5.2   Consumer response to changed producer amino acid and fatty acid composition

Additionally to changes in overall protein concentrations, leaf quality may be affected by free amino acids or mass-produced storage proteins. The composition of free amino acids changes markedly as a result of nitrogen deposition (Perez-Soba et al. 1994, Huhn and Schulz 1996), and this may be significant to herbivores, as these amino acids are taken up in the digestive tract without the costs of digestion, or even act as the major uptake route of bioavailable N, for instance in phloem and

xylem feeding organisms. Nectar-feeding insects have been shown to incur lowered survival due to increased concentrations of amino acids in nectar due to nitrogen deposition, most likely because only a few amino acids become more abundant and hence the nectar amino acid composition changes (David et al. 2019). Also, the digestion of a single mass-produced storage protein, like Rubisco, may be less effective compared to a mixture of proteins, if the single protein is difficult to access or has difficult bonds to break by digestive enzymes (e.g. dependent on food type the digestibility coefficient for cysteine varies between 58% and 87% in poultry; Leeson and Summers 2001). The most likely route for nitrogen deposition to cause amino acid deficiencies in animals, seems a generally altered protein synthesis in plants. Rare but essential amino acids in plants (such as tryptophan and methionine) which are used in catabolic and anabolic pathways throughout the food chain, may become deficient in top predators.

Amino acid limitations have been reported in Sparrowhawks *(Accipiter nisus)* in forests of the Veluwe, but far less so in their prey, e.g. Great tits *Parus major* (van den Burg et al. 2014). Current research indicates that in Great tit nestlings, feather development is limited by amino acids, especially by methionine (Van den Burg, unpublished data), but this is a much less severe fitness effect compared to egg and nesting failures in Sparrowhawks. Experimentally introduced half-grown caterpillars (the prey of the Great tits) develop well on oak (*Quercus robur*) trees in this forest, but show high mortality during pupation, possibly explaining low moth population sizes on oak trees on strongly acidified, nutrient poor soils under chronic high N deposition (van den Burg et al. 2014). Caterpillar mortality itself is not related to amino acid or element deficiency, but may be related to non-protein nitrogen (NPN) toxicity. Oak tree leaves show an overall reduction in amino acid levels (van den Burg et al. 2014), which is unrelated to overall leaf nitrogen content, indicating an increase of NPN. Current hypothesis is that, as nitrogen availability is high, leaf NPN compounds increase, possibly affecting primary and higher order consumers through mechanisms of amino acid deficiency or NPN toxicity.

Blooms by phytoplankton, notably diatoms and cyanobacteria, can lead to reduced food quality, including fatty acid deficiencies, thiamine deficiency, and toxic effects of NPN, notably non-proteionomic amino acids. Blooms by phytoplankton are promoted by eutrophication, including nitrogen availability. Some bloom forming phytoplankton, including diatoms, contain little HUFA's (Gomes et al. 2016), and their blooms may thus have consequences for higher trophic levels. This is particularly a problem in animals adapted to HUFA rich diets, as they have often lost the capability to produce HUFA's from PUFA precursors. For many other animals, HUFA's are semi-essential, but the performance of these animals (including humans) is much better if there is a dietary source of HUFA's and/or DHA (Twining et al. 2016). Enrichment by N has also been associated to reduced PUFA content of phytoplankton communities, and led to reduced abundances of consumers (i.e. *Daphnia*) (Trommer et al. 2019), indicating cascading negative effects of N enrichment through shifts in the macronutrient composition.

5.5.3 Consumer response to changed vitamin content in producers

In paragraph 2.5.2, we discussed the lack of relevant papers in the literature survey on vitamin availability in relationship with nitrogen deposition (see Appendix 1). Since the general effects of micronutrient deficiencies have been presented in chapter 3, they will not be further discussed here, because they are often not specifically linked to or reported as a result of increased N-deposition. We conclude that there exists a knowledge gap when considering possible causal links between nitrogen deposition and changed micronutrient availability and subsequent effects at higher levels in the food chain.

5.6 N deposition effects on antifeedants and phytotoxins

5.6.1 Effects on producer quality

Nitrogen deposition exerts effects on the producer organisms defence system and thus producer quality for consumers. In aquatic systems, phytoplankton cellular levels of N containing compounds such as saxitonin were shown to strongly depend on nitrogen availability and decrease when

nitrogen is limited (Van de Waal et al. 2009, Van de Waal et al. 2013, Gobler et al. 2016). With excess nitrogen under phosphorus limitation, cellular levels of these compounds were also shown to increase (Van de Waal et al. 2013, Frenken et al. 2017). Thus, nitrogen containing toxins directly depend on the nitrogen availability, and generally increase when phytoplankton cells contain a relative excess of nitrogen. Moreover, a number of toxic and/or allelopathic compounds in harmful algal blooms species were shown to be inversely related to nutrient availabilities. Possibly, this is because these are C based compounds, which were shown to generally increase under N and P limited conditions (Van de Waal et al. 2014). Enhanced nutrient enrichment, including N deposition, may thus possibly limit synthesis of some toxins and allelochemicals, with potential consequences for the producing harmful algal species and phytoplankton community composition (Granéli et al. 2008).

In aquatic as well as terrestrial systems, an general rule is that with increasing N input, N containing allelochemicals may be elevated, but levels of carbon based antifeedants are reduced (Hofland-Zijlstra and Berendse 2009, Bandau et al. 2015, Campbell and Vallano 2018, Nybakken et al. 2018). In a recent meta-analysis of N deposition experiments in terrestrial ecosystems, C-based allelochemical concentrations (polyphenolics and phenolics) were found to signicicantly decrease with increased N supply (Sun et al. 2020; **Figure 19**). The proposed mechanism responsible for this effect is the increased carbon-use for growth instead of defense when nitrogen becomes less limiting (Nybakken et al. 2018). It seems however unknown how this interaction plays out at the condition of a strong excess of nitrogen relative to other nutrients, in which trees show decreased protein synthesis and growth (van den Burg et al. 2014) . Although the general rule (more N, less C-based defenses) thus seems to be supported by recent studies, significant variation on the level of sites, species, foliage age, and compounds is likely to exist (Hamilton et al. 2001) and interactions with other soil factors such as soil P and K availability and soil acidity and buffer status are also highly likely to act as modulating factors in predicting N deposition effects on C based allelochemicals.
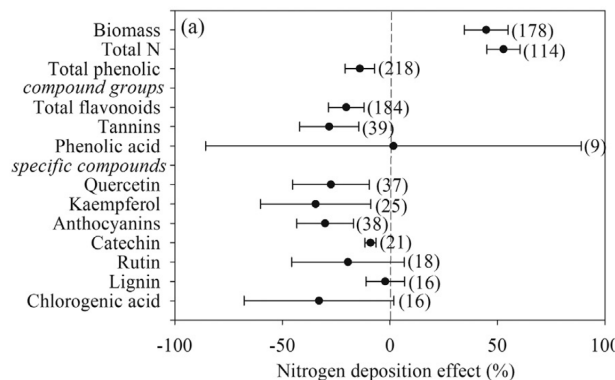
Figure 19. *Effect of simulated N deposition on plant biomass and the concentrations of total N and multiple phenolic compounds in the plant. Every symbol represents the mean response ratio of different variables and the error bar represents the 95% confidence intervals (CIs). Sample size is displayed beside each bar in parentheses. Mean bar values >0 indicated a positive effect of simulated N deposition on the variable, a negative effect is shown when bar value is <0 (Sun et al. 2020).*

Figuur 19. *Effect van gesimuleerde N-depositie op plantenbiomassa en de concentraties van totaal N en verscheidene fenolverbindingen in de plant. Elk symbool vertegenwoordigt de gemiddelde responsratio van verschillende variabelen en de foutbalk vertegenwoordigt de 95% betrouwbaarheidsintervallen (CI's). De steekproefgrootte wordt naast elke balk tussen haakjes weergegeven. Gemiddelde waarden> 0 duidden op een positief effect van gesimuleerde N-depositie op de variabele, een negatief effect wordt getoond wanneer deze waarde <0 is (Sun et al. 2020).*

In current year needles of Norway spruce the general rule seemed to hold, but previous year needles showed an increase in some stilbenes and tannins (Nybakken et al. 2018). Hofland-Zijlstra and Berendse (2009) found a decrease in phenolics, but not in tannins in heather *Calluna vulgaris*.

These studies indicate that interactions between nutrient supply and allelochemical concentration exist, but that the identity of the limiting nutrient, as well as the mechanism (dilution effect vs changes in production rate) determine the outcome of altered nutrient supply. Although the above effects on antifeedant levels have been reported in literature, most studies do not include bioassays of herbivore (insect) performance, leaving the ecological significance of increased or decreased antifeedant levels obscure. For example, Campbell and Vallano (2018) show that at high nitrogen treatment (soil nitrate) and (hence) reduced phenolics, caterpillars in their experiment still performed poorly, illustrating that a reduction in an antifeedant not necessarily promotes insect growth, as positive effects of lower phenolics may have been nullified by an increase of N-based defensive compounds or due to nutritional constraint.

5.6.2   Consumer response to changed producer allelochemical concentrations

In terrestrial systems, C-based allelochemical concentrations have been shown to be negatively correlated with N-supply. In a N fertilization experiment with douglas fir, increasing N supply resulted in reduced concentration of phenolics (Joseph et al. 1993). Growth of Gypsy moth (*Lymantria dispar*) caterpillars feeding on high N (and hence low phenolic) containing needles) was significantly higher. However, to what degree lower phenolics attributed to the increased growth remained unclear since it coincided with higher N content of the needles which on itself proved beneficial for caterpillar growth. For instance, increased larval developmental rate of winter moth (*Operophtera brumata*) caterpillars (a generalist species feeding on woody plants) were shown to be only resulting from increased food quality (here lower C:N ratio of *Calluna vulgaris*; Kerslake et al. 1998), since phenolic content did not change significantly in N treated plants in this study.

In aquatic systems, many algae produce toxic secondary metabolites often (but not always) having a negative effect on grazers, including immobilization, reduced growth, and mortality (e.g. see Turner 2006, Ger et al. 2016). Toxins were shown to follow largely stoichiometrically predictable patterns, where various N-rich alkaloids and peptides reduce with N limitation and increase under P-limitation, while a C-rich toxins tend to increase with N and P limitation (Van de Waal et al. 2014). Although such nutrient dependent shifts in toxins may alter consumer performance, this has not yet been tested. The relationships between toxin contents and grazers performance is complicated by a number of factors, including evolutionary adaptation of grazers in their detoxification, and/or the ability of some zooplankton species to selectively feed more edible less toxic phytoplankton (Turner and Tester 1997, Turner 2006, Ger et al. 2016).

5.7 Effects on higher order consumers

5.7.1   Effects of changed prey stoichiometry, diversity and density on predators

Until recently it was generally accepted that predator body composition matches those of prey, and that predators were primarily limited by prey availability rather than quality. However, food selection behavior for balancing nutrient intake as reported for many herbivores (Behmer 2009) also has been found in several predacious invertebrate species (Mayntz et al. 2005, Raubenheimer et al. 2007, Jensen et al. 2011, Jensen et al. 2012), suggesting that protein:carbohydrate imbalances between prey and predators may also have an evolutionary and ecologically relevant effect on predator fitness, albeit much less profound compared to primary producers and herbivores. Intake regulation studies have found evidence for stronger intake regulation of carbohydrates than for protein (Raubenheimer et al. 2007, Jensen et al. 2012), which is opposite to the general rule found in herbivore intake regulation studies (Behmer 2009).

As for macronutrients, stoichiometric imbalances have also been reported. For invertebrate predators, tissue N content has been found to be significantly higher than herbivores, with predators having on average 15% more N than herbivores (Fagan et al. 2002, Gonzalez et al. 2017), (but see Hambäck et al. 2009). This may be logically explained in terms of supply; predatory organisms feed on food sources substantially richer in N than the food sources of herbivores. Predators may have a higher N requirement due to higher necessary investments in muscle tissue to enable them to forage and catch prey. However, due to the obligate higher

mobility rates of predators compared to herbivores, predators have relatively higher C demands for respiration, which might explain the stronger intake regulation towards sufficient carbohydrate intake (Raubenheimer et al. 2007, Jensen et al. 2012). Lemoine et al. (2014) however also found that predators had significantly lower N:P ratios than herbivores, and that potential P limitation in predators was as high as potential N (or C) limitation.

Higher trophic level effects of altered producer nutrient quality can be substantially dampened by consumer homeostasis. If a high degree of nutrient homeostasis is obtained, prey species nutrient content and/or ratios will remain the same, irrespective of host plant quality and hence cannot affect predators feeding on these species. In a tritrophic study, Kagata and Ohgushi (2007) concluded that prey C:N ratio could not explain an observed increase in the RGR of a coccinelid predator *Aiolocaria hexaspilota*, while at the same time, host plant N content was found to have increased the RGR of its prey species *Plagiodera versicolora*. The degree of C:N homeostasis that is realized by *P. versicolara* (Kagata and Ohgushi 2006) resulted in no change in prey C:N ratio between treatments, and hence no response in predator growth rate or fitness. Conversely, high predator density can impact the stoichiometry of lower trophic levels via negative feedback mechanisms. Griffith and Grinath (2018) found that leaf N increased and leaf C:N decreased under presence of spider intraguild predation, facilitating sap sucking herbivore density, which in turn altered plant nutrient quality.

Predator prey availability and composition can however be substantially altered as a result of changed plant quality, as interspecific differences in the optimal intake ratio of C, N and P leads to changes in herbivore community composition under conditions of changed producer stoichiometry. Lee et al. (2017) found a reduced species diversity of herbivore invertebrates two years after (very high) N-fertilization and subsequent lowered C:N ratio of *Quercus crispula* leaves, and concluded that this effect may be due to species differences in optimal nutrient C:N intake ratio, altering the ratio of reproductive success between species and promoting dominance of a few species, resulting in a reduced Shannon diversity. Studies that address these effects on field situations, under N deposition relevant scenarios are however extremely scarce. Haddad et al. (2000) found a reduced herbivore and predator species richness response to increased yearly N addition experiments in grasslands. Vogels et al. (2017) found a reduced herbivore density and accompanying reduced predator species richness on heathland sites with increasing N:P ratios, due to N deposition mediated shifts in C:N:P stoichiometry. Higher trophic levels can thus be indirectly affected by reduced prey diversity and/or availability resulting from N-deposition mediated changes in producer stoichiometry. An increase in producer N:P ratio has been found to negatively affect the density of (generally low N, high P; Fagan et al. 2002, Woods et al. 2004) Lepidoptera larvae in heathlands (Vogels 2013, van den Burg and Vogels 2017). This decrease in density of Lepidoptera larvae has been shown to be a major contributor to the gradual extinction of the last remaining relict population of Black Grouse (*Lyrurus tetrix*) in the Netherlands (Ten Den and Niewold 2012, Vogels 2013, Ten Den and Niewold 2017, 2018, Vogels 2019).

5.7.2 Increased Ca limitation in insectivorous birds

The effects of Ca availability on fitness has been studied most extensively in insectivorous bird species, often in the context of increased acidification rates and concomitant decrease in Ca supply (Mahony et al. 1997). In acidic aquatic streams, dipper (*Cinclus cinclus*) populations laid fewer eggs, and showed a delayed egg-laying period compared to circumneutral streams. Moreover, Ca levels in the blood serum of adult dippers were lower and alkaline phosphatase enzyme levels in the serum were higher in acidic stream habitats (Ormerod et al. 1991). These differences could be linked functionally to the prey item composition, with much lower numbers of Ca rich prey items such as fish as snails present in acidic stream habitats (Ormerod et al. 1991). In terrestrial ecosystems, Schlender et al. (2007) showed a significant effect of soil acidification on the species assemblage of 14 common forest songbirds, negatively affecting song thrush *(Turdus philomelos)*, Nuthatch *(Sitta europea)*, displacing blackbird *(Turdus merula)* territories to forest edges, and positively affecting robin *(Erithacus rubecula)*, chaffinch *(Phylloscopus collybita)* and coal tit *(Parus ater)* territorial densities. Ramsay and Houston (1999) found no evidence for Ca deficiency as

determining factor in blue tit (*Parus caeruleus*) eggshell deficiencies, even under highly acidified site conditions, indicating that Ca deficiencies are not always the main driver for this observed phenomena. Different Ca uptake strategies between bird species (as suggested by Schlender et al. 2007) may explain the observed differences in species sensitivity to reduced Ca availability.

Ca deficiencies are most likely to occur in the sandy soils of the Netherlands, which are poor in mineral nutrients by nature. As such these soils are also the most prone to acidification and loss of plant nutrients such as Ca. Reports of Ca deficiencies in birds on the Veluwe date back to the nineteen fifties (source: H. van Balen); many forests are on former heathlands and drift sands which have long lost most Ca-bearing minerals. This is not only due to acidification, but also resulting from centuries of former land use, involving sod-cutting of heather vegetation and forest soil, and due to nutrient transport towards more fertile soils flanking the region (Bouwer 2008, Vervloet 2015). Recent reports of Ca deficiencies in tits (*Parus spp)* from the Veluwe indicate continued acidification in an area wherein which formerly such Ca deficiencies were not occurring (Van den Burg 2017b).

6 A test of hypotheses: butterfly trends under contrasting N deposition history in the Netherlands

6.1 Introduction

Butterflies constitute a well-studied group of herbivorous insects that are known to be sensitive to nitrogen availability in their environment. Although it is obvious that species depending on eutrophic environments, such as nettle-feeding butterflies, largely benefit from nitrogen enrichment (Kurze et al. 2017), the proportion of species that could be negatively affected is much larger (Wallis de Vries and van Swaay 2017, Kurze et al. 2018). Fischer and Fiedler (2000) were among the first to bring forward evidence that challenged the nitrogen limitation hypothesis for insect herbivores. Recently, N deposition has indeed been found to impact pollinator (bees and butterflies) species richness negatively (Carvalheiro et al. 2020), suggesting that soil eutrophication also impacts higher trophic levels. In this study however, no mechanistic understanding of the observed N deposition driven pollinator decline was provided. In this chapter, following earlier exploratory analyses by Wallis de Vries and van Swaay (2013), we aim to fill this gap by presenting an analysis of butterfly distribution and population trends in relation to nitrogen enrichment, taking butterfly species traits into account.

From the literature review in chapter 0, in particular par. 2.2 and 2.4, we will test two main hypotheses. The first hypothesis is that food plant specialists are more vulnerable to changes in N:P (and protein:carbohydrate) ratios than generalists, since they tend to occupy a narrower stoichiometric niche than generalists (par. 2.2.3, 2.4.4). Especially species from oligotrophic environments then should show declining population trends under raised N deposition, since shifts in producer N:P ratio would occur faster and to a greater degree than in meso-eutrophic environments (par. 2.2.3). In contrast, food plant generalists and species not restricted to oligotrophic environments, but also reproducing in meso- and eutrophic habitats should either not be affected by raised N deposition or even benefit from higher N availability.

The second hypothesis is that species feeding on species with comparatively low N:P ratios, i.e. herbaceous species (2.2.2.6, 2.2.3) should experience greater population declines under raised N deposition than species depending on host plants with generally higher N:P ratios, i.e. graminoids, dwarfshrubs or woody plants.

Below, we have tested these hypotheses on the basis of, firstly, long-term trends in the distribution of butterfly species in the Netherlands since 1950 and, secondly, from population trends of selected species since 1992. Within the Netherlands, we focused on species reproducing in semi-open oligotrophic habitats on Pleistocene sandy soils, i.e. habitats that are most vulnerable to impacts of nitrogen deposition. The long-term analysis of distribution change consists of trends at country-level. The population trend analysis involved changes in local abundance across various levels of nitrogen deposition on poorly buffered soils on the one hand, and difference in trends between poorly and fairly buffered soils at a similar nitrogen deposition level on the other hand.

6.2 Methods

6.2.1 Post-1950 distribution trends

A selection of 34 species of butterflies was compiled that are native to the Netherlands and potentially reproduce on Pleistocene sandy soils in semi-open oligotrophic habitats, i.e. the soil type most affected by nitrogen deposition (Appendix 2). This excludes species restricted to eutrophic habitats. Species traits were collected on nitrogen or productivity optimum and range (after Ellenberg nitrogen values of their habitat and larval host plants; Oostermeijer and van

Swaay (1998), Aguirre-Gutiérrez et al. (2016), Wallis de Vries and van Swaay (2017), host plant species and type (herbs, graminoids, dwarfshrubs, woody plants or generalist host use encompassing several plant families) and whether larvae had a thermophilous microclimatic preference or not (Bink 1992, Wallis de Vries 2014). Trends in the distribution of host plant species were obtained from www.verspreidingsatlas.nl.

Ellenberg nutrient values reflect nutrient availability in ecosystems and have been shown to present a reasonable proxy for nitrogen availability (Schaffers and Sýkora 2000, Wamelink et al. 2002). On this basis, species nutritional niches were classified as:

- indifferent (8 species) if either the nutrient niche breadth, i.e. the range of at least 80% occurrence probability, was broader than 3 Ellenberg units or the species exhibited no significant nutrient optimum,
- mesotrophic (13 species) if the optimum Ellenberg nutrient value was between 3 and 6 with a niche breadth of at most 3 units,
- oligotrophic (13 species) if the species' Ellenberg nutrient value was below 3 and niche breadth was 1 unit or less.

In line, with expected species traits, species with low nutrient indicator values have a lower reproductive potential and longer generation times (Wallis de Vries and van Swaay 2017).

For all species, post-1950 distribution trends were available as the percentage of 5x5 km squares in which the species currently occurs (2011-2017) relative to the number of squares in the period 1940-1960 (Van Swaay 2019). In an ANOVA, the variation in distribution change was explained as a function of nutritional niche, host plant type (herbaceous vs. non-herbaceous) and microclimatic preference. Residuals were checked for normality.

Species with an indifferent nutritional niche were expected to show a neutral to increasing distribution trend, whereas species restricted to oligotrophic habitats were expected to decline (**Table 9**). For species with herbaceous host plants, typically associated with lower N:P ratios, we expected declining trends in contrast to species with other non-herbaceous host plants. In addition, we included species microclimatic preference, as species with a preference for warm spring microclimates may be expected to suffer from microclimatic cooling under nitrogen deposition (Wallis de Vries and van Swaay 2006). However, as this trait is also linked to an association with oligotrophic habitats, we did not expect it to be of more than marginal significance. For some species, the expected response is uncertain, due to contrasting combinations of nutritional niche and hostplant use, e.g. generalist species from oligotrophic habitats could be expected to benefit from their flexibility in host plant use but suffer from their adaptation to an oligotrophic environment.

Table 9*. Expected response of butterfly species trends as a function of nutritional niche and host plant use.*

Tabel 9*. Verwachte respons van trends van vlindersoorten als functie van voedingsniche en waardplantgebruik.*

Nutritional niche	Host plant use	Expected response
indifferent	generalist and non-herbaceous	neutral to increase
	herbaceous	uncertain
mesotrophic	non-herbaceous	uncertain
	herbaceous	decline
oligotrophic	generalist	uncertain
	herbaceous and non-herbaceous	decline

6.2.2 Post-1992 population trends

Trends in butterfly abundance on Pleistocene sandy soils were obtained from the Dutch Butterfly Monitoring Scheme for the period 1992-2018. In this analysis, we focussed on 10 reasonably widespread species with common host plants where possible effects of nitrogen deposition on plant quality can be expected. We carried out two types of analyses. We first examined differences in trends in regions with different long-term nitrogen deposition on soils with poor buffering capacity. The second analysis dealt with variation in trends between soils with either poor or fair buffering capacity at similar long-term nitrogen deposition.
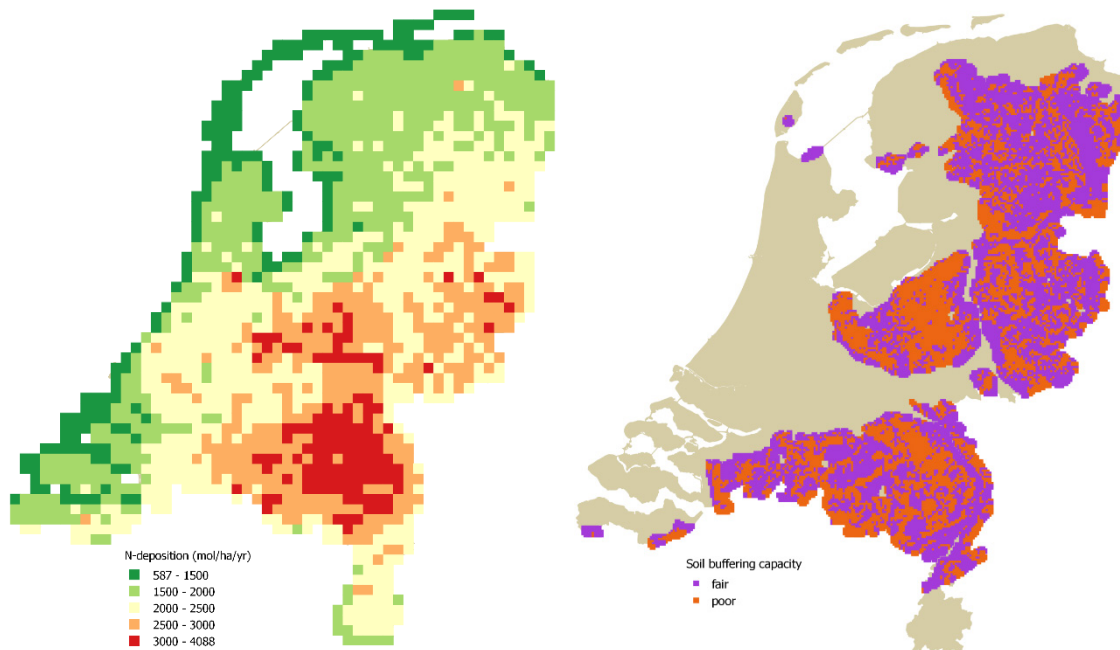
Figure 20. *Spatial distribution across the Netherlands of the long-term average nitrogen deposition at 5x5 km resolution (left; data from RIVM) and soil buffering capacity in the region of Pleistocene sandy soils at 1x1 km resolution (right; derived from NHI data portal) (see text for explanation).*

Figuur 20. *Ruimtelijke spreiding over Nederland van het lange termijn gemiddelde van stikstofdepositie op een 5x5 km resolutie (links; data van RIVM) en bodembuffercapaciteit in de regio van Pleistocene zandbodems bij 1x1 km resolutie (rechts; afgeleid van NHI data portal) (zie tekst voor uitleg).*

Long-term (1981-2017) data on annual deposition of reduced and oxidised nitrogen from RIVM were used to estimate the long-term average nitrogen load at 5x5 km resolution (**Figure 20**); data from 2006 onwards were at 1x1 km resolution, which were used to arrive at an approximation for a finer resolution. Subsequently, regions were divided in five levels of long-term nitrogen deposition, from which four (i.e. above 1500 mol N/ha/year) were relevant for the Pleistocene sandy soils.

Soil buffering capacity in the sandy soils region (**Figure 20**) was estimated as poor or fair at 1x1 km resolution for 17094 km-squares in three steps. First, PAWN soil units (Wösten et al. 1988) (Wösten et al., 1988) at 25 m resolution, accessed by data.nhi.nu/, were assigned to poor or fair buffering capacity, with the 'poor' units formed by fine or coarse sandy soils low in loam content as well as peat soils and more loamy soils or soils with a build-up of organic matter from agricultural legacies ('enkeerdgronden') were assigned as 'fair'. Squares were assigned as 'poor' or 'fair' depending on the largest area covered in the square. Secondly, to account for raised buffering capacity through recent agricultural influences, 1137 km-squares with more than 5 ha of herb-rich grassland (mapped as unit N12.02 on the portal http://bron.portaalnatuurenlandschap.nl/ from 'poor' soil units, were included as 'fair' units. Thirdly, to account for other possible. e.g. anthropogenic, sources of buffering capacity a further 28 km-squares with at least 4 characteristic plant species from *Nardus*-rich plant communities were also included as being of 'fair' buffering

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capacity, as *Nardus*-rich plant communities have been demonstrated to be particularly sensitive to nitrogen deposition (after van der Zee et al. 2017).

Trend analysis was performed using the software TRIM (Pannekoek and Van Strien 2005), a Poisson GLM programme to produce annual indices and linear trends. Species were only considered suitable for analysis if there were at least 5 sites per stratum with a time series covering at least 15 years (Appendix 3). Two types of trend analyses were carried out. The first determined trend differences between regions varying in level of nitrogen deposition on poorly buffered soils.

We expected species with an indifferent nutritional niche not to show clear trends with increasing nitrogen deposition: increases could well be found if species are nitrogen-limited despite their occurrence over a broad range of nutrient availability, but detrimental impacts of eutrophication (e.g. on flower resources) might occur at the highest levels of nitrogen deposition. For species with a mesotrophic to oligotrophic niche, we expected greater declines at higher nitrogen deposition levels. For the oligotrophic niche, the trends of four rare species could only be analysed for two broad nitrogen deposition strata. Finally, we did not expect food plant generalist species to show different trends in response to nitrogen deposition, in contrast to food plant specialists, where nitrogen deposition was expected to have stronger adverse effects on population trends.

The second analysis examined differences between soil regions at the same level of nitrogen deposition. This comparison was made for two nitrogen deposition levels for which most data were available. Here, we expected species with a mesotrophic or oligotrophic nutritional niche to show greater declines on poorly buffered soils on the one hand. Species with an indifferent nutritional niche, on the other hand, might also suffer more from eutrophication on soils with greater buffering capacity.

6.3 Results

6.3.1 Post-1950 distribution trends

Long-term changes in butterfly distribution were significantly different across species nutritional niches (indifferent: +63±17, mesotrophic: –55±13, oligotrophic: –82±7; $F_{2,33}$=32.70; p<0.0001) and to a lesser extent by host plant type (herbaceous: –76±10, non-herbaceous: –22±15; $F_{1,32}$=7.7;p=0.0095), but there was no significant effect of microclimatic preference (p=0.39).
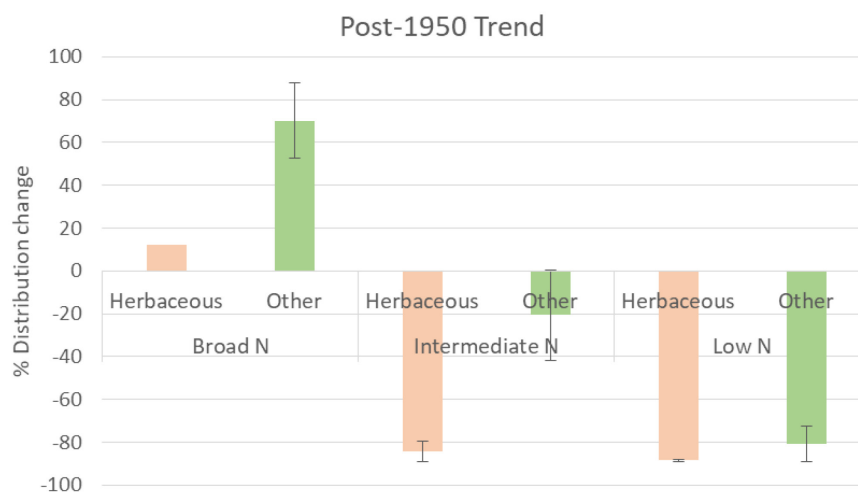
Figure 21. *Mean post-1950 distribution change (± 1 S.E.) of butterfly species of difference nutritional niches and host plant use, expressed as the relative change in occupied 5x5 km squares (after Van Swaay 2019).*

Figuur 21. *Gemiddelde distributieverandering na 1950 (± 1 S.E.) van vlindersoorten met verschillende voedingsniches en waardplantgebruik, uitgedrukt als de relatieve verandering in bezette 5x5 km-vierkanten (naar Van Swaay 2019).*

Species with indifferent nutritional niches all experienced range expansions, whereas species with oligotrophic and mesotrophic niches showed significant declines, and this was most severe in oligotrophic species and mesotrophic species with herbaceous host plants (**Figure 21**).

Species with herbaceous host plants showed a less favourable trend than species with other host plant types. The host plants from 8 of the 10 species with herbaceous host plants also showed declines in their distribution, in contrast to species with non-herbaceous host plants with only one out of 24 species having a declining host plant. Trends in host plant availability thus may have been a driver of decline besides changes in plant quality. The three species exclusively feeding on dwarf shrubs also experienced severe declines of 80% or more.

6.3.2 Effects of nitrogen deposition on post-1992 population trends

Population trends in relation to nitrogen deposition were all considered in areas of poor soil buffering capacity, where the impacts on plant quality are expected to be most severe. Only species with common host plants were considered, in order to avoid possible confounding influences of changes in host plant availability.

For four species with indifferent nutritional niches, population trends showed a different response across regions varying in nitrogen deposition (**Figure 22**). The anticipated optimum response was detected in three species, with strong declines at the lowest and highest level of nitrogen deposition in *P. tithonus* and *T. lineola*; for *L. phlaeas* trends qualitatively also reflected an optimum response, but the variation in trends was too large to reveal differences between nitrogen deposition levels. Only *M. jurtina* showed a different response pattern with a significantly positive trend at lower nitrogen deposition in contrast to stable trends at higher deposition levels.

***Figure 22**. Mean annual slope of post-1992 population trends (± 1 S.E.) at different levels of N deposition for butterfly species with indifferent nutritional niches; M. jurtina, P. tithonus and T. lineola are graminoid feeders, L. phlaeas is an herbacous host plant specialist.*

***Figuur 22**. Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) bij verschillende N-depositie niveaus voor vlindersoorten met brede (indifferent) voedingsniches; M. jurtina, P. tithonus en T. lineola zijn graseters, L. phlaeas is gespecialiseerd op kruidachtigen.*

Three of the four examined mesotrophic species (**Figure 23**) have graminoid host plants. *O. sylvanus* showed an optimum response in population trend across nitrogen deposition levels, similar to most of the species with an indifferent nutritional niche. *T. sylvestris* had negative

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population trends at both deposition levels which allowed data analysis. *C. pamphilus* showed the most positive trends at highest levels of nitrogen deposition. *L. tityrus* was the only examined mesotrophic species with an herbaceous host plant. As expected, for a species with an herbaceous host plant that does not tolerate a broad nitrogen range, it showed a strong decline at high N, contrasting with a positive trend at a N deposition level of 2000-2500 kg N/ha/yr.

As expected, the two species restricted to oligotrophic habitats with generalist host plant use showed no significant variation in population trends across regions differing in nitrogen deposition (**Figure 24**).
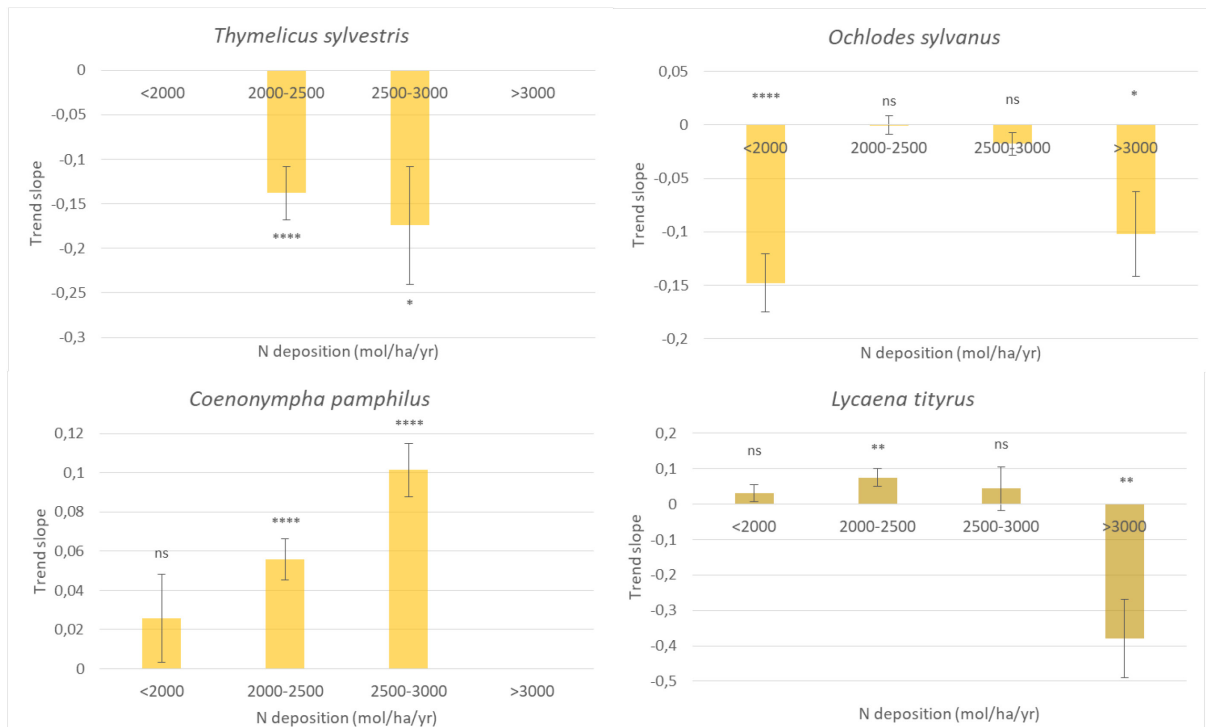
Figure 23. *Mean annual slope of post-1992 population trends (± 1 S.E.) at different levels of nitrogen deposition for butterfly species with mesotrophic nutritional niches; all three species shown in orange are graminoid feeders, L. tityrus is an herbaceous host plant specialist.*

Figuur 23. *Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) bij verschillende N-depositie niveaus voor vlindersoorten met mesotrofe voedingsniches; alle drie de oranje soorten zijn graseters, L. tityrus is gespecialiseerd op kruidachtigen.*

Figure 24. *Mean annual slope of post-1992 population trends (± 1 S.E.) at different levels of nitrogen deposition for butterfly species restricted to a oligotrophic range; both species shown have a generalist host plant use strategy.*

Figuur 24. *Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) bij verschillende N-depositie niveaus voor vlindersoorten met oligotrofe voedingsniches; beide getoonde soorten zijn waardplantgeneralisten.*

Although data availability for oligotrophic species with more specific host plant use was limited due to their rare occurrence, two species with graminoid host plants and one with a dwarfshrub host plant showed significantly greater declines at higher levels of nitrogen deposition (**Figure 25**), although this concerned only a single site for *B. aquilonaris* and for *C. tullia*. For one further species in this group, the grass-feeding *Hesperia comma,* no significant trend difference between nitrogen levels was found (overall trend −0.041±0.021, n=60 sites, not shown in figure).
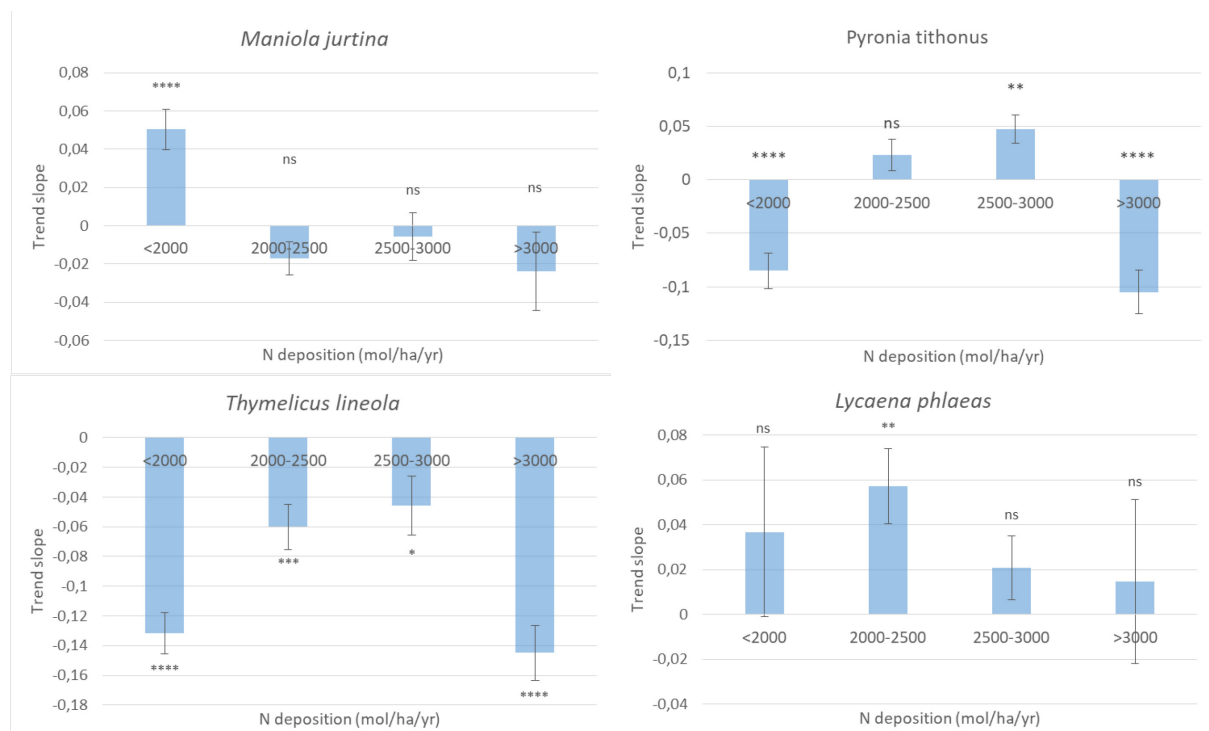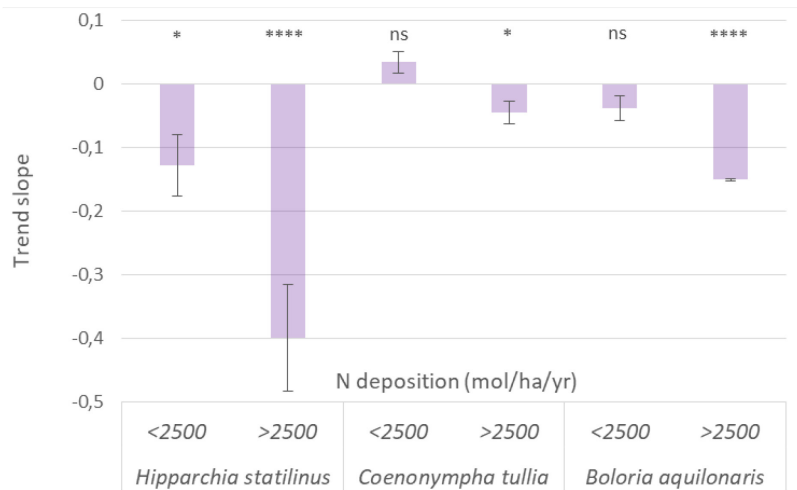

Figure 25. *Mean annual slope of post-1992 population trends (± 1 S.E.) at different levels of nitrogen deposition for three butterfly species restricted to an oligotrophic nutritional niche; H. statilinus (n= 13 sites) and C. tullia (N=13 sites) feed on graminoids, B. aquilonaris (n=5 sites) is specialised on the ericoid Vaccinium oxycoccos.*

Figuur 25. *Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) bij verschillende N-depositie niveaus voor vlindersoorten met oligotrofe voedingsniches; H. statilinus (n= 13 sites) en C. tullia (N=13 sites) zijn graseters, B. aquilonaris (n=5 sites) is gespecialiseerd op de heideachtige Vaccinium oxycoccos.*

6.3.3 Influence of soil buffering capacity on post-1992 population trends

Population trend differences between soils with fair or poor buffering capacity were examined at two levels of nitrogen deposition. Three out of four species with indifferent nutritional niches showed a tendency for more positive trends on poorly buffered soils than on soils with higher soil buffering capacity (**Figure 26**). This could indicate an effect of N enrichment and increase of food plant quality for these species in these soil types (i.e. increased N content results in higher plant quality for these species). However, the difference was only significant for *P. tithonus* and for *T. lineola* at the higher nitrogen deposition level. Again, *M. jurtina* was the exception in this group, with worse trends on poorly buffered soils, the difference being significant at the lower level of nitrogen deposition.

The mesotrophic species showed variable patterns in their trends at different soil buffering capacity (**Figure 27**). *T. sylvestris* showed a less negative trend at fair buffering capacity when long-term nitrogen deposition was in the range of 2000-2500 mol N/ha/yr, but the difference was not apparent at higher nitrogen deposition. For *C. pamphilus,* trends were more positive on poorly buffered soils at both levels of nitrogen deposition, which could indicate a eutrophication effect. This was only the case for *O. sylvanus* at the lower level of nitrogen deposition, but at higher nitrogen deposition the difference was reversed, with a slightly better trend on fairly buffered soils. No significant pattern emerged for the herbaceous host plant specialist *L. tityrus.*

The two species oligotrophic habitats with generalist host plants, as expected, showed no significant differences in population trends on soils with different buffering capacity (**Figure 28**). Data were insufficient to allow analyses on soil buffering for species with more specialised host plant use.
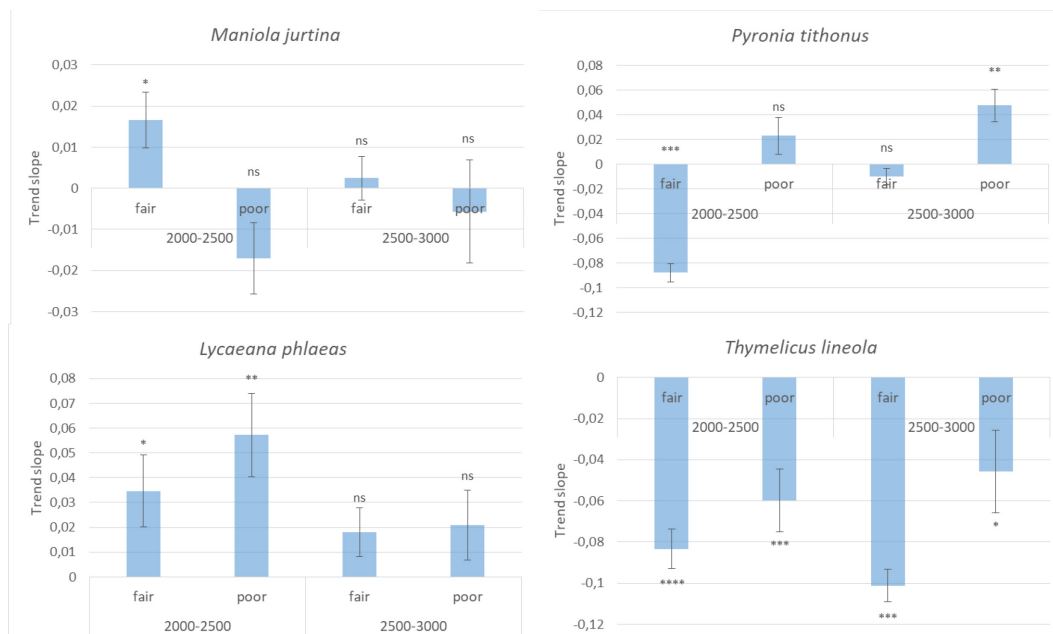
Figure 26. *Mean annual slope of post-1992 population trends (± 1 S.E.) on soils of fair or poor buffering capacity at similar N deposition levels (2000-2500 or 2500-3000 mol N/ha/yr) for butterfly species with indifferent nutritional niches; M. jurtina, P. tithonus and T. lineola are graminoid feeders, L. phlaeas is an herbaceous host plant specialist.*

Figuur 26. *Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) op bodems met een redelijke versus slechte buffercapaciteit onder vergelijkbare N-depositieniveaus (2000-2500 of 2500-3000 mol N/ha/jaar) voor vlindersoorten met brede (indifferent) voedingsniches; M. jurtina, P. tithonus en T. lineola zijn graseters, L. phlaeas is gespecialiseerd op kruidachtigen.*
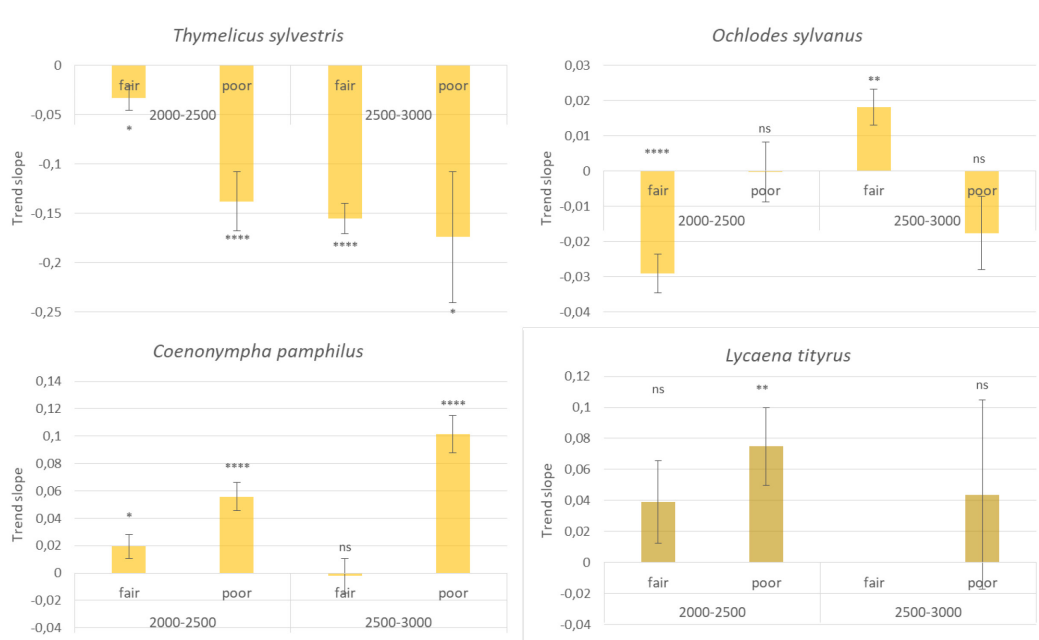
Figure 27. *Mean annual slope of post-1992 population trends (± 1 S.E.) on soils of fair or poor buffering capacity at similar N deposition levels (2000-2500 or 2500-3000 mol N/ha/yr) for butterfly species with mesotrophic nutritional niches; all three species shown in orange are graminoid feeders, L. tityrus is an herbaceous host plant specialist.*

Figuur 27. *Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) op bodems met een redelijke versus slechte buffercapaciteit bij vergelijkbare N-depositieniveaus (2000-2500 of 2500-3000 mol N/ha/ jaar) voor vlindersoorten met mesotrofe voedingsniches; alle drie de oranje soorten zijn graseters, L. tityrus is gespecialiseerd op kruidachtigen.*
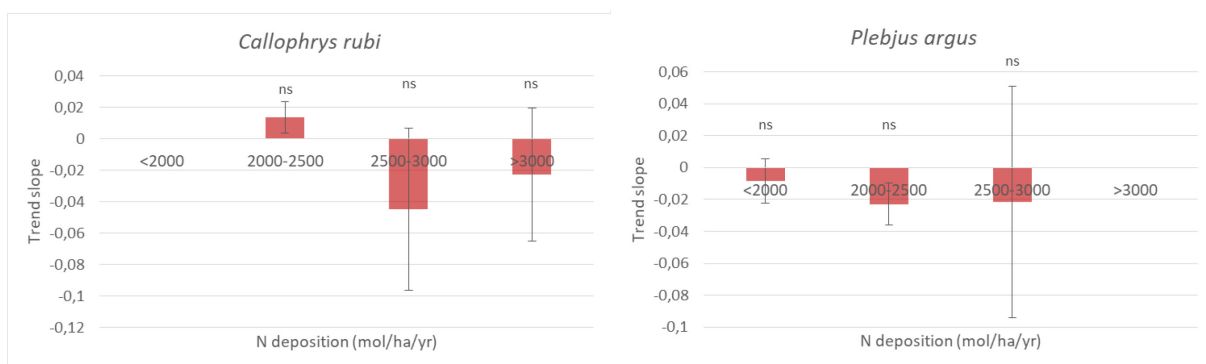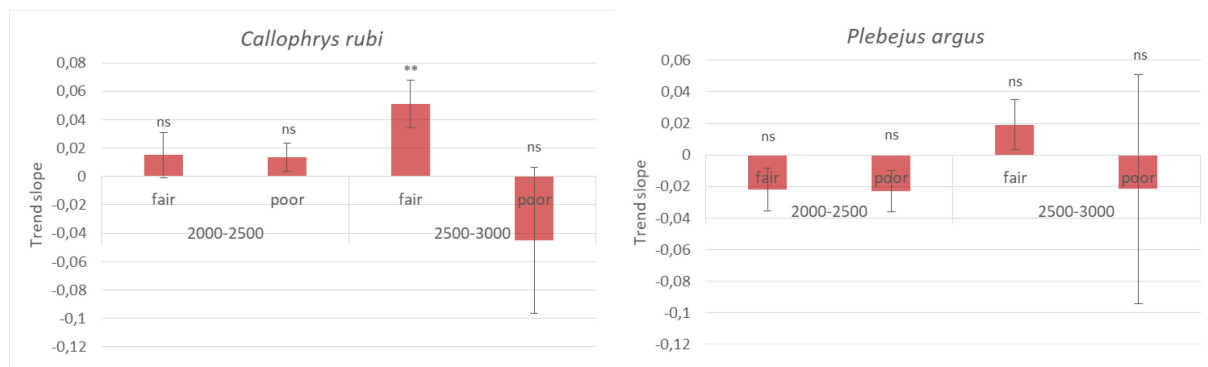
Figure 28. Mean trend slope of post-1992 population trends (± 1 S.E.) on soils of fair or poor buffering capacity at different levels of nitrogen deposition (2000-2500 or 2500-3000 mol N/ha/yr) for butterfly species restricted to an oligotrophic nutritional niche; both species shown have a generalist host plant use.

Figuur 28. Gemiddelde jaarlijkse helling van populatietrends na 1992 (± 1 S.E.) op bodems met een redelijke versus slechte buffercapaciteit bij vergelijkbare N-depositieniveaus (2000-2500 of 2500-3000 mol N/ha/ jaar) voor vlindersoorten met oligotrofe voedingsniches; beide soorten zijn waardplantgeneralisten.

6.4 Discussion and conclusions

Long-term trends in butterfly distribution of species differing in nutritional niche and host plant use on nutrient-poor sandy soils completely agreed with *a priori* expectations (for nine species with uncertain expectations, the trends were evenly distributed between decline and neutral to increase (5 and 4 species, respectively). Species with an indifferent niche in relation to nitrogen availability generally increased in distribution, whereas species restricted to low-nitrogen environments generally strongly decreased. Furthermore, species decrease was greater for species with herbaceous host plants. These decreases are both due to the loss of habitat by the disappearance of host plants, such as *Succisa pratensis, Thymus serpyllum, Viola* species, *Gentiana pneumonanthe, Lotus corniculatus* and *Potentilla erecta.* Many of these species are associated with Nardion of Junco-Molinion plant communities that are threatened by nitrogen enrichment (Jansen 2000, van der Zee et al. 2017). For species with declining host plants, decreases in distribution may still partly be due to changes in host plant quality, but this effect cannot be disentangled from their shrinking availability. Nevertheless, species from oligotrophic habitats without declining host plants predominantly showed substantial declining distributions as well.

For declining species with non-declining host plants, changes in plant quality could be a main factor driving the loss of suitable habitat. This concerns 12 species:

- three species feeding on woody plants: *Agriades optilete, Boloria aquilonaris* (both on dwarfshrubs)*, Satyrium ilicis* (oak)
- five graminoid-feeding species: *Coenonympha tullia, Hesperia comma, Heteropterus morpheus, Hipparchia semele, Hipparchia statilinus* and *Thymelicus sylvestris*
- two foodplant generalists: *Plebejus argus, Plebejus idas* (extinct during the 1980s)*,*
- one herb-feeding species: *Lycaena phlaeas.*

Seven of these, highlighted in red above, have shown ongoing declines in abundance since 1992.

The analysis on species-specific population trends since 1992 revealed complex patterns in relation to nitrogen deposition and soil buffering capacity. These are probably due in part to the inevitable noise in the estimation of local habitat conditions at the butterfly monitoring sites in terms of long-term nitrogen deposition levels and soil buffering capacity. Still, some relevant (correlative) patterns seem to emerge from the analysis.

In relation to long-term nitrogen deposition, four out of seven species for which nitrogen impacts were not expected to be detrimental, showed indications of an optimum response of population

trends. For these species of typically mesotrophic habitats, the less favourable trends at the lower end of nitrogen deposition could reflect suboptimal conditions in nutrient-poor environments. At high nitrogen deposition, the negative trend could be due to (severe) eutrophication. Whether certain nitrogen deposition levels ultimately lead to declines in these species is also determined by other factors, such as land use and climate. In the context of this study, it is mainly the relative differences in trends between regions with different levels of nitrogen deposition that should be considered.

A group of five species showed more favourable population trends at long-term nitrogen deposition levels below 2500 mol N/ha/yr. In agreement with expectations, this included the host plant specialist from mesotrophic habitats *L. tityrus* as well as tentative evidence from limited data for three species restricted to oligotrophic environments *H. statilinus, C. tullia* (both graminoid-feeding) and *B. aquilonaris* (a specialist feeding on *Vaccinium oxycoccos*). The exception was the graminoid-feeding *M. jurtina*, a species with a supposedly indifferent nutritional niche, for which the more positive trend at a long-term nitrogen deposition levels below 2000 mol N/ha/yr warrants further investigation. This species has been found to be sensitive to changes in nectar quality and quantity in agricultural areas (Lebeau et al. 2016), which may indicate a limitation through adult rather than larval resources. *T. sylvestris* experienced similarly strong population declines at both the 2000-2500 and 2500-3000 levels of nitrogen deposition. As this species has been linked to nitrogen-sensitive Nardion-grasslands (van der Zee et al., 2017), it could well be that it only thrives at much lower levels of nitrogen deposition. For *L. tityrus,* the negative response at high nitrogen deposition is in line with the reduced survival of caterpillars under nitrogen enrichment, as found by Fischer and Fiedler (2000)and Kurze et al. (2018) under experimental conditions. For *B. aquilonaris,* a negative response to high nitrogen deposition was inferred by Turlure et al. (2013) by the lower growth rates and survival of caterpillars fed on host plants originating from a region with high nitrogen deposition (Drenthe, NL) as compared to a low deposition area (Ardennes, B).

The absence of variation in population trends across nitrogen deposition levels for the two generalist species from oligotrophic habitats, *C. rubi* and *P. argus,* could be due to a combination of their flexible host plant use and their preference for wetter habitats, where acidifying effects from excess nitrogen are better buffered through groundwater seepage. Indeed, population trends of *P. argus* are more favourable in wet than in dry habitats (Wallis de Vries and Oteman 2019) and this difference could then override the overall effect of nitrogen deposition.

As to effects of soil buffering capacity, indications of a surprising negative effect of soil buffering capacity were found for six species with a mesotrophic to indifferent nutritional niche. This could be due to grass encroachment on better buffered, i.e. richer soils that are more susceptible to eutrophication than acidification.

The only evidence for a positive effect of soil buffering capacity was found for *T. sylvestris* at a long-term nitrogen deposition level of 2000-2500 mol N/ha/yr*,* which is in line with its preference for nitrogen-sensitive Nardo-Galion grasslands (see above). The absence of this difference at higher nitrogen deposition might be due to the positive effect of soil buffering being overridden by nitrogen excess.

The absence of effects of soil buffering on the trends of the two species restricted to oligotrophic habitats, *C. rubi* and *P. argus,* might again be due to their generalistic feeding strategy and confounding effects of groundwater seepage in the wetter habitats that they prefer (see above).

6.4.1   Summary conclusions
Observed effects on long-term distribution changes and current population trends of the six butterfly species that were expected to be negatively affected by nitrogen deposition were found to be consistent for *Boloria aquilonaris, Coenonympha tullia, Hipparchia statilinus,* and *Thymelicus silvestris,* although the evidence rests on a limited number of sites*.* Negative trends of the herbaceous host-plant specialist *Lycaena tityrus* at high nitrogen deposition warrant further

investigation, as this species has also been found to suffer from excess nitrogen in controlled experiments. For *Hesperia comma,* the population trends were inconclusive.

For the four species with an indifferent nutritional niche, which were expected to be benefitting from or tolerant to nitrogen deposition, three showed evidence of an optimum response in population trend to nitrogen deposition (*Lycaena phlaeas, Pyronia tithonus* and *Thymelicus lineola*). This could indicate a negative effect of eutrophication at the highest levels of nitrogen deposition. The more favourable trends of the supposedly nitrogen-indifferent species *M. jurtina* at lower nitrogen deposition and on better buffered soils, might indicate an impact of nitrogen deposition on adult food resources, c.q. nectar quality and availability, rather than larval resources.

Finally, among the further four species with an uncertain response expectation, the foodplant generalists *Callophrys rubi* and *Plebejus argus* showed no significant difference between either nitrogen deposition levels or soil buffering capacity. This may be explained by the flexibility in their host plant use in combination with a preference for wetter, less acidified habitats. For the other two, *Coenonympha pamphilus* and *Ochlodes sylvanus,* population trends indicate a benefit from nitrogen at lower levels of deposition, but vulnerability to eutrophication at the highest levels, as for the three abovementioned species with an indifferent nutritional niche.

In conclusion, long-term distribution trends in relation to nitrogen deposition could be satisfactorily predicted on the basis of a combination of nutritional niche and host plant use. Post-1992 population trends of selected species with stable host plant occurrence were more difficult to explain in relation to nitrogen deposition. A number of species from mesotrophic habitats appeared to show nitrogen limitation at lower N deposition, but negative effects of eutrophication at high N deposition. Two foodplant generalists showed stable trends throughout. Finally, the decline of four (and possibly five) species was in line with possibly detrimental changes in host plant quality, which deserves further investigation.

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7 Resumé and general conclusions

7.1 Effects of N deposition on soil and water

N deposition will leads to significant N enrichment of naturally oligotrophic ecosystems in both the terrestrial and aquatic biomes. In aquatic systems, enrichment effects may also have significant ecological implications in eutrophic waters when nitrogen is temporally limiting. In the longer term soil C:N ratio has been shown to considerably decrease. At the same time, soil and water N:P ratio has consistently been reported to increase. Soil acidification is also an important secondary effect of N deposition. It leads to loss of ANC, a lower soil pH, increased leaching of base cations (Ca^{2+}, K^+, Mg^{2+}) and increased concentrations of potentially toxic metals (e.g. Al^{3+}), a decrease in nitrification rate and in litter accumulation. These changes in soil and water chemistry, buffering and relative abundances of elements may have severe consequences for the chemistry of the vegetation, and may cascade towards higher trophic levels.

7.2 Effects of N deposition on producer quality and consumer performance

As a result of N deposition induced changes in soil and water chemistry, producer chemistry can also change substantially. Especially under oligotrophic, weakly buffered or acidic conditions, the concentration of N in producer tissue increases without a concomitant increase in tissue C or P content, resulting in strong deviations in producer C:N:P stoichiometry. This increased uptake of N is often accompanied by an increase in N-rich free amino acids, especially when ammonium is the dominant form of inorganic N. Numerous studies have found a significant decrease in producer tissue C:N ratio and a concomitant increase in N:P ratio under increased N deposition. Consumer responses to N deposition mediated changes in elemental stoichiometry are however not univocally positive or negative. Reports of increased consumer species response to reduced C:N ratio as well as reports of decreased consumer species response exist, the latter most often then linked to increased N:P ratio.

This discrepancy in consumer response can be attributed to 1) differences in C:N:P stoichiometry between studies (i.e. a reduction in C:N ratio without changes in N:P ratio would easier result in consumer fitness increase in contrast to study conditions showing reduced C:N but concomitant increased producer N:P ratio); 2) differences in the consumer species specific TER and 3) the degree of food plant specialization of consumers. The direction of consumer response to changed C:N:P stoichiometry depends on whether this results in a shift towards the consumer specific TER or away from it, which in turn depends on the specific consumer physiology and/or life history tactic. In general, such changes in producer C:N:P ratio could however result in an overall decrease in consumer community species richness, as it promotes outbreak events of a small subset of species, and concomitant declines in other species, depending on the species specific TER for C:N:P. However, there is currently still a lack of specific N deposition related studies that address effects of N deposition using this paradigm, especially in terrestrial habitats.

In parallel to changed ratios in the major elements C, N and P, increased N deposition has been repeatedly shown to have significant implications on the N:element ratio, especially for K and Mg and often also Ca in producers, effectively lowering the elemental concentration of these elements in producers, including forests, heathlands and fen ecosystems. For trace elements, these effects are reported much less, but existing reports suggest that these elements can either become limiting or are reduced due to growth dilution effects. The precise working mechanism, the degree as well as the specific element that becomes limited is however dependent on specific ecosystem

properties such as soil and water type, buffer status, temperature, moisture, etc., and should be investigated in an ecosystem specific context. In a N deposition specific context, Ca deficiency in vertebrates has most often been reported as a result of increased soil acidification. The mechanism behind reduced Ca-availability is however not directly related to Ca availability in producer tissue, but by the acidity of the soil, in which prey species bearing Ca rich exoskeletons are becoming increasingly scarce with increasing acidity. In aquatic systems, some evidence exists that other elements (Fe, Zn) may as well become limiting for consumers, which has also been evidenced in terrestrial (invertebrate) consumers for Na, Mg, K and trace elements (see paragraph 2.3.3). Until now, however, no specific studies have been performed that link N deposition induced reductions in elemental availability in producers to reduced performance of consumers.

Also for proteins and carbohydrates, there exists a discrepancy between the amount of publications in animal nutrition studies (i.e. nutritional geometry studies) and those found in N deposition studies. In the latter, articles usually report producer C:N ratio as proxy for carbohydrate:protein ratio. In terms of carbohydrate and protein content, this is probably not viable in many cases, especially when also considering the effect of N deposition on the ratio of free amino acids and N rich storage protein rather than functional protein in producers. Under the framework of nutritional geometry, it is hypothesized that an overall increase in protein content will often result in a decreased total intake of carbohydrate (as well as other nutrients, such as P, K, Mg, etc). Also, there are reports of lowered protein content in producers under increased N deposition, under highly acidic conditions. These reports of protein content in producers not increasing despite increased N concentration under increased N deposition further indicate that considering producer C:N ratio as crude measure of protein content is not viable in producer-consumer interactions in field situations. We thus conclude that there exists a knowledge gap in the effects of N deposition on the availability of protein and carbohydrate and it subsequent effect on consumer performance via intake regulatory mechanisms.

As proteins are made of amino acids, N deposition may have profound effects on the amino acid makeup of producer protein. When total 'crude protein' ingested is regulating total food intake in consumers, an increase in producer storage protein can induce a consumer shortage in essential amino acids, specifically those that are less (or not) used in these storage proteins. This effect may be especially affect consumers that strictly regulate their intake of protein (see paragraph 2.4.3). Essential fatty acid shortages may also arise earlier under increased protein content of producer tissue under strict protein intake regulation, regardless of the fatty acid content of producers. Intake regulatory mechanisms thus have to be taken into account when considering N deposition effects on 'total carbohydrate:protein ratios', but also when considering the specific essential carbohydrate and amino acid makeup of producers. The few terrestrial and aquatic studies that exist do indicate that increased N deposition can negatively affect first and higher order consumers via altered producer amino acid, essential fatty acid and/or essential vitamin composition. Here too, a major knowledge gap on the severity and exact working mechanisms still exists.

Perpendicular to N deposition altering the nutritional composition of producers, N deposition can influence the composition and concentration of allelochemicals in producers, which generally harm consumers with increasing concentrations. Especially under naturally oligotrophic conditions, N based allelochemical concentration tend to increase under increasing N supply, while C based allelochemicals decrease under increasing N supply. Whether this general rule also holds under highly increased N supply due to chronic N deposition is however questionable, as growth would no longer be limited by N supply, reducing the allocation of C into growth and subsequently increasing C allocation to plant defense molecules. In general, when increasing N supply results in lowered C based allelochemicals, food quality for herbivores will increase, especially for poorly adapted generalist feeders (see par. 2.6 and 3.5), which might further explain the increased occurrence of plague events of single herbivore species under increased N deposition. However, increases in N based allelochemical concentration under increased N deposition would result in the opposite effect, especially for generalist consumers.

The exact outcome of N deposition on allelochemical producer consumer interactions are thus dependent on the producers specific defense strategy, consumers specific adaptations and regulatory mechanisms for plant defense compounds and the accompanying effect of altered nutritional content of producers on consumer fitness. Here too, the lack of sufficient N deposition related case studies does not allow for general conclusions to be made.

Although intake regulation for C:N:P and/or proteins and carbohydrates also exist in predators, N deposition effects on prey stoichiometry are likely to be dampened by intake regulation of the prey items. Hence, higher trophic levels are expected to be much less dependent on N deposition mediated changed first order consumer nutrient ratios, but more so on N deposition mediated changes in prey diversity and abundance. The number of studies that address the impact of N deposition on higher order consumers are however very scarce. The existing studies however indicate that the impact of such changes on predator populations can be substantial. For vertebrate predators, acidification induced reduction in Ca availability (through reductions in Ca rich prey items) has often been reported and shown to affect bird populations. Higher order consumers can thus be affected by N deposition via reduced prey availability and/or variability, which can partially be linked to a reduced intake of essential elements normally provided by a group of essential prey species.

In testing the hypotheses formulated in chapters 0 and 3 on host plant specialization and nutritional niche specialization of consumers (host plant specialists of oligotrophic habitats being most prone to show negative effects of altered producer quality, and host plant generalists of eu- to mesotrophic habitats being least prone to show negative effects), long-term population trends of butterflies in relation to nitrogen deposition were performed and showed that these trends could indeed be satisfactorily predicted on the basis of a combination of a species nutritional niche and host plant use, although exceptions were also found. The results of this study were however not always conclusive; incorporating other relevant species traits (mobility, temperature niches, etc.) and/or a more detailed dataset regarding host plant characteristics, N deposition and soil chemistry are recommended to further test the hypothesis of life history tactic dependent vulnerability to changed producer quality.

7.3 General conclusion and hypotheses

The number of N deposition related studies addressing producer-consumer relationships with respect to the various nutritional quality aspects is limited, which complicates generalization regarding overarching mechanisms, directions of change or effect size of N deposition effect on producer quality. In general, the amount of evidence found in literature decreases with the degree of complexity of the specific nutritional framework applied, but even in the most overarching framework Ecological Stoichiometry, there are still major knowledge gaps in the underlying mechanisms. Moreover, the interaction of other environmental factors with the effect of N deposition on consumer fitness seems poorly understood.

Despite the knowledge gaps, and based on the available studies and existing frameworks, we hypothesize that species occupying narrow niches in oligotrophic, poorly buffered or acidic habitats will likely encounter reduced fitness in response to increased N deposition, which imposes an additional threat to the sustainable persistence of populations of those species under chronically increased N deposition.

For the quality and protection of N2000 protected habitats and associated species, this hypothesis has major implications, since many of the species listed as characteristic for oligotrophic and/or poorly buffered N2000 habitats fall within this category. We propose that a deeper knowledge on the exact working mechanisms and severity of impact are necessary in order to implement a sound N emission reduction policy as well as to devise measures that are able to mitigate these adverse effects.

Based on the proposed mechanisms and hypotheses formulated in chapter 0 and 3, together with the current state of knowledge reviewed in chapter 0, several testable hypotheses and directions for future research can be formulated. In order of increasing complexity (not priority), these are:

- Whether a consumer species will benefit or suffer from increased N deposition depends on the effect of N deposition on the elemental ratio of producers, and the direction of this change in relation to the consumers threshold elemental ratio (TER) or nutrient intake target (see paragraphs 2.2.2; 2.4.3; 3.1; 3.3; 5.2 and 5.4).
- Overall consumer species richness will decrease under increased N deposition via fitness increases in a limited subset of species and concomitant fitness decline of another subset of species. This will be mechanistically regulated via the species specific TER of consumers (2.2.2; 3.1; 5.2 and 5.7).
- Consumer species highly adapted to a single or small set of related producer species are most likely to encounter fitness penalties resulting from increased N deposition, due to a smaller optimal stoichiometric intake bandwidth for growth and/or stronger intake regulation for protein and carbohydrate (see paragraphs 2.2.2; 2.4.3; 3.1; 3.3; 5.2 and chapter 0).
- In contrast, consumer species that employ a highly generalist consumption strategy are most likely to encounter fitness increases resulting from increased N deposition, due to a broader optimal stoichiometric intake bandwidth, accompanied by fitness increases resulting from decreased plant allelochemical content (see paragraphs 2.2.2, 2.4.3, 2.6, 3.1, 3.3, 3.5, 5.2 and chapter 0).
- Producer content of Na, Mg, K and trace elements, such as Cu and Co, have recently been shown to influence herbivore populations more than previously assumed. Leaching of these elements due to acidification (caused by N deposition) may affect consumer populations to a much stronger degree than currently acknowledged and thus require further study (see paragraphs 2.3, 3.2 and 5.3).
- In N deposition related studies, the generally adopted C:N ratio for determining producer quality is regarded here as an unsuitable means of measuring producer quality. Effort should be made in quantifying the effect of N deposition on total protein and carbohydrate, as well as the specific protein and carbohydrate makeup. According to nutritional geometry theory, these nutrient types are the major currencies used in consumers in determining nutritional quality and in regulating the intake of nutrients (see paragraphs 2.4, 3.3 and 5.4).
- Besides the effect of N deposition on C:N ratios and the protein and carbohydrate content and composition, the specific building blocks of protein and carbohydrate (amino acids and fatty acids) have been shown as key drivers determining consumer fitness. However, assessment of these biochemicals are currently highly underrepresented in ecological literature. Since there exists a high degree of variation in species specific requirements for amino acids and fatty acids, ample knowledge on the specific amino acid, (vitamin) and fatty acid requirements are a prerequisite for understanding the effect of N deposition on food quality and consumer fitness (see paragraphs 2.5, 2.7, 3.5, 3.6, 5.4 and 5.6).
- The effect of N deposition on nutritional quality does not only result from bottom up effects on producer quality, but is also strongly modulated by the consumer species' (evolutionary) adaptations to cope with inadequate food quality (summarized in paragraph 2.7). We therefore recommend using a trait-based or life history tactic-based approach in studying the effect of increased N deposition on consumer response.

7.4 Outstanding questions

Besides the aforementioned hypotheses and research directions, we also came across a number of more fundamental outstanding questions, which we summarized here. These can be categorized into two major themes: 1) questions related to basic understanding of food quality aspects in consumers and 2) questions related to the impact of N deposition on food quality.

Basic understanding of food quality

- What is the role of trace elements in determining food quality and what are the implications of ionomic mismatch between producers and consumers?
- Is there a relationship between micronutrient and elemental composition as indicators for food quality?
- How big is the role for micronutrients in determining producer quality for consumers in ecosystems?
- What is the role of behavioral changes in dealing with nutritional mismatches, and to what extent does this depend on feeding strategy?
- To what extent interacts the consumer holobiont towards mismatches in food quality?

Impacts of N deposition

- What is the impact of N deposition on micronutrient composition of producers and what are the consequences for consumers?
- Are there producer-consumer interaction driven trade-offs in N deposition related shifts in food quality, palatability and toxicity?
- To what extent does the impact of N deposition on food quality depend on the trophic state of an ecosystem, and do these impacts vary across season?
- What are the impacts of N deposition dependent shifts in producer species composition on food quality for generalist consumers?
- Are there fundamental differences in the pathways and/or severity of N deposition effects on the nutritional composition of producers for consumers between aquatic and terrestrial biomes?
- How, and how often will food quality impacts of N deposition on consumers cascade to higher trophic levels?

Part 3: Sensitivity of Natura 2000 habitat types and associated species

8 Sensitivity estimation

In this chapter we will provide an estimation of the sensitivity of specific habitats and species for increased nitrogen deposition, with respect to changed food quality. First and foremost the authors wish to stress that this estimation is meant as a tool in addressing the need for altered restoration measures, intensity and methods and in devising a priority agenda for further research, solely based on the topic of changes in food quality. As N-deposition mediated changes in food quality is only one of many possible pathways involved in altering consumer communities (see **Figure 3** and Nijssen et al., 2017), this assessment cannot be used as argumentation to alter or define the 'general sensitivity' of a given ecosystem or associated species to increased N deposition. The estimation will be made using the knowledge of ecosystem and organismal functioning and subsequent trophic effects reviewed in chapters 0 through 0, combined with results from habitat specific effect studies from literature, when available.

Here, we first present a decision tree that is used in this sensitivity estimation, which provides the logic and argumentation behind the resulting estimated sensitivity to increased N deposition, in terms of nutrient quality.

8.1 Underlying decision tree

8.1.1 N2000 habitat types

The effects of increased nitrogen deposition on soil and water quality (further referred to as substrate quality) and subsequent effects on plant nutritional quality and consumer performance will differ between habitat types. In chapters 0 through 0 the effects and underlying mechanisms are described and substantiated with literature, which can be used to analyse the vulnerability of different habitat types for N deposition. These factors are here summarized in a decision tree to standardize this analyses. The different branches in the decision tree apply for terrestrial as well as aquatic habitats, unless indicated otherwise. It should be noted that the decision tree provides a rather simplified depiction of the natural coherence and interactions between these mechanisms. The decision tree is built upon four specific habitat characteristics:

1) <u>Natural availability of N and P in substrate</u>: Different from the general mechanisms in plant or vegetation ecology, the nature of the limiting element does not define the sensitivity of a given ecosystem to changed nutritional quality under increased N deposition. From a vegetation perspective, 'pure' N limited habitats are most vulnerable to increased N deposition as under these conditions, increased N availability leads to the most strong increase in growth, resulting in strong shifts in vegetation composition. From a plant CNP stoichiometric perspective however, changes in producer growth also has implications for the relative content of these elements, through dilution effects in case of highly stimulated growth (i.e. 'pure N limitation') or through increased nutrient concentration in case of moderate or no growth benefit (i.e. strong P or N/P co-limitation). Therefore, all oligotrophic to mesotrophic habitats, irrespective of the nature of the limiting element are vulnerable to changed CNP stoichiometry under increased N loading. Especially vulnerable are oligotrophic, N-limited and N/P co-limited habitats, as relative changes will be most profound under such conditions (N limitation: increase in plant tissue N, strong dilution of plant tissue P though increased growth; N/P co-limited: strong increase in plant tissue N, some dilution of plant tissue P through moderate growth).
Note that P-limited systems where P is present as calcium phosphate ($Ca_3(PO_4)_2$) can switch to N limited or N/P co-limited systems under influence of acidification of the

substrate, especially when ANC recharging dynamic processes (inundation, aeolian dynamics) are inhibited.

In aquatic conditions, temporal changes in the nature of limitation are also important to assess: in normally non N limited lakes and other large softwater bodies, N limitation can temporarily occur in summer when N is mostly captured in plant and microorganism biomass. N deposition can undo this temporarily N limitation, promoting blooms of poorly edible algae and thereby strongly altering food web dynamics. Terrestrial eutrophic habitats however will be less vulnerable especially when plant growth is limited by other factors like dynamic processes, grazing, mowing, etc..

2) <u>Acid Neutralizing Capacity (ANC):</u> N deposition has an acidifying effect on the habitat substrate; pH drops, Ca^{2+}, Mg^{2+} and K^+ are mobilized and can become less available through leaching in terrestrial habitats, while free Al^{3+} increases. Habitats with weekly buffered and acidic substrate will react faster and more severe than habitats with a high buffer capacity. Habitats with highly acidic substrate are also highly vulnerable as under these conditions, N is mainly available as ammonium which can lead to greatly increased plant tissue N or to changes in plant elemental content via toxicity effects on plant physiology. Note however that the duration of N deposition is also important in this process, as under chronic high N deposition also well buffered ecosystems will eventually encounter these adverse effects of acidification.

3) <u>Dynamic processes</u>: frequent influence of dynamic processes such as inundation (with river of brook water) or erosion and accumulation of substrate due to wind or flowing water are often 'recharged' with nutrients and ANC are less vulnerable to N deposition than stable habitats.

4) <u>Hydrology</u>: Buffer capacity of habitats under influence of groundwater are continuously recharged, especially when they are fed with seepage water. Dry and wet habitat which are predominantly fed by rain water are more vulnerable for effects of N deposition.

These factors are used to build a decision tree for estimating the vulnerability of different habitat types for N deposition in a way that changes in food quality for consumers can occur in these habitats (**Figure 29**).

8.1.2 N2000 associated species

Most research is focused on the effects of N deposition on soil, vegetation or habitat changes, while much less is known about the specific effects of nitrogen deposition on animal species (but see chapter 0 and 0 in this report). Even less research is focused on the effects of N deposition on specific species which are protected under N2000. In the review of Nijssen et al. (2017), a 'change in producer quality' is one out of seven possible mechanisms of N deposition driven effects, but effects of changed producer chemistry on consumer performance is addressed as a major knowledge gap. Also in this report, in which the whole route from changes in substrate and plant chemistry to shifts in consumer performance are described in single steps, only few publications can be cited in which causal relations and dose-effect responses on a single species is analysed (see chapter 0).
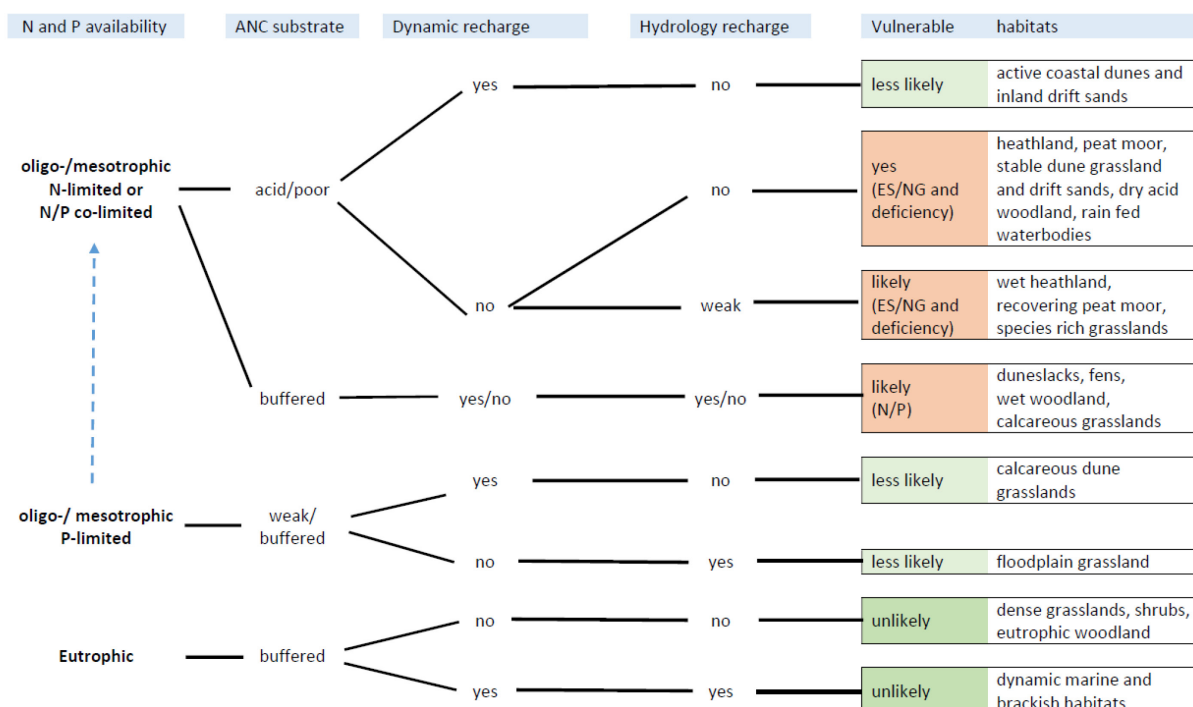
Figure 29. *Simplified decision tree used in the vulnerability assessment of habitat types for changes in food quality for consumers due to increased N deposition. Assessment is based on 1) natural availability and (co)limitation of N and P , 2) Acid Neutralizing Capacity (ANC) and the input of extra nutrients and recharging ANC by 3) regularly occurring dynamic processes or by 4) extruding groundwater or inundation with surface water. Vulnerability noted with ES/NG means relevant effects on in C:N:P stoichiometry (Ecological Stoichiometry) or protein:carbohydrate ratios (Nutritional Geometry) are expected; vulnerability noted with 'deficiency' means changes in availability of (essential) micronutrients or (trace) elements are expected. Note that P-limited systems can switch to N limited or N/P co-limited systems under influence of acidification of the substrate (blue arrow), especially when ANC recharging dynamic processes (inundation, aeolian dynamics) are inhibited. Habitats listed are given as example.*

Figuur 29. *Vereenvoudigde beslisboom gebruikt bij de kwetsbaarheidsbeoordeling van habitattypen op veranderingen in voedselkwaliteit voor consumenten als gevolg van verhoogde N-depositie. De beoordeling is gebaseerd op 1) natuurlijke beschikbaarheid en (co)limitatie van N en P, 2) zuur neutraliserend vermogen (ANC) en de toevoer van extra nutriënten en/of het opladen van ANC door 3) regelmatig voorkomen van dynamische processen of door 4) het uittreden van grondwater of overstroming met oppervlaktewater. Kwetsbaarheid aangemerkt met ES / NG geeft aan dat relevante effecten op C:N:P stoichiometrie (ecologische stoichiometrie) of proteïne:koolhydraat ratio's ('nutritional geometry') wordt verwacht; kwetsbaarheid aangemerkt met 'deficiency' betekent dat veranderingen in de beschikbaarheid van (essentiële) micronutriënten of (sporen) elementen wordt verwacht. Bedenk dat P-gelimiteerde systemen kunnen omslaan naar N-gelimiteerde of N/P co-gelimiteerde systemen onder invloed van verzuring (blauwe pijl), vooral wanneer dynamische processen die het zuurneutraliserend vermogen aanvullen (inundatie, eolische dynamiek) worden geremd. De hier vermelde habitats zijn als voorbeeld gegeven en niet uitputtend.*

However, in reviewing the current frameworks and paradigms used in animal ecological research (chapter 0), several hypotheses have been formulated that provide links between species traits, feeding guilds, allelochemical interactions and food specialization to the degree of vulnerability to N deposition induced changes in producer quality. Parallel to the analyses for habitat types, a decision tree model can be made to estimate the effects of increased nitrogen deposition on animal species. This tree is based on 7 traits or characteristics:

1) <u>Rank in food web</u>: First order consumers seem to be more vulnerable for changes in plant quality than second consumers and higher trophic predators. However, top-predators can be vulnerable for N deposition mediated declines in prey availability and/or diversity and for deficiency of specific essential (micro)nutrients, especially in the reproduction period when large investments in offspring are needed.

2) <u>Feeding guild</u>: Within the first order consumers, whole plant part feeders are more vulnerable than browsers (foraging on specific plant parts) and suckers (foraging solely on cell content or plant sap flow, which exhibit strong endosymbiotic relationships with amino acid producing micro-organisms).

3) <u>Specialisation on food source (I)</u>: Specialist (mono- or oligophage species) show higher vulnerability for changes in relative availability of major elements (i.e. elemental stoichiometry) and/or protein to carbohydrate ratio (i.e. nutritional geometry) compared to generalist (polyphagous species). However, generalists are often more vulnerable to increases in antifeedants, where adapted specialists can often cope much better with these components and thus show lower fitness costs under increasing allelochemical concentrations.

4) <u>Food source (II)</u>: Grasses and woody plants are in general less nutritious compared to herbaceous plants and fluctuate more in N/P ratio throughout the growing season. Grass and woody plant adapted species are therefore more adapted to changes in food quality and are thus hypothesized to be less vulnerable for changes in nutritional quality.

5) <u>Food source and antifeedant (III)</u>: Generalist feeders feeding on naturally low N plants (i.e. high C:N plants) are hypothesized to show most often a strong increase in fitness (outbreaks), especially in conditions in which plant tissue C:N is dropped considerably from a relatively high natural ratio, and concomitantly plant tissue C-based antifeedants (phenolics) concentration are also lowered. Such outbreak events seem to be most pronounced in species feeding on woody plants.

6) <u>Mobility:</u> Species that are less mobile (small home range in relation to body size) are more sensitive to changes in food quality than species that are mobile (large home range) which can explore a larger and more diverse food availability (an also often exhibit higher intake plasticity for nutrients).

7) <u>Phylogeny:</u> Species belonging to phylogenetic lineages that are reported to have relatively low tissue N content and relatively high tissue P content (i.e. having a low tissue N:P ratio) are more vulnerable to changes in plant tissue N:P ratio than species from lineages with higher tissue N:P content. Based on our literature review, these are species belonging to Diptera and Lepidoptera. This phylogenetic characteristic is probably however highly variable between different species and is thus only used here as an additional factor in assessing a given species vulnerability (i.e. only given weight when other conditions for vulnerability are also met).

Factors 1 to 4 are used to build a decision tree that estimates in general the vulnerability of Natura 2000 associated animal species for changes in nutrition quality of plants due to increased N deposition (**Figure 30**). Factor 5 to 7 are used for fine tuning of this vulnerability.
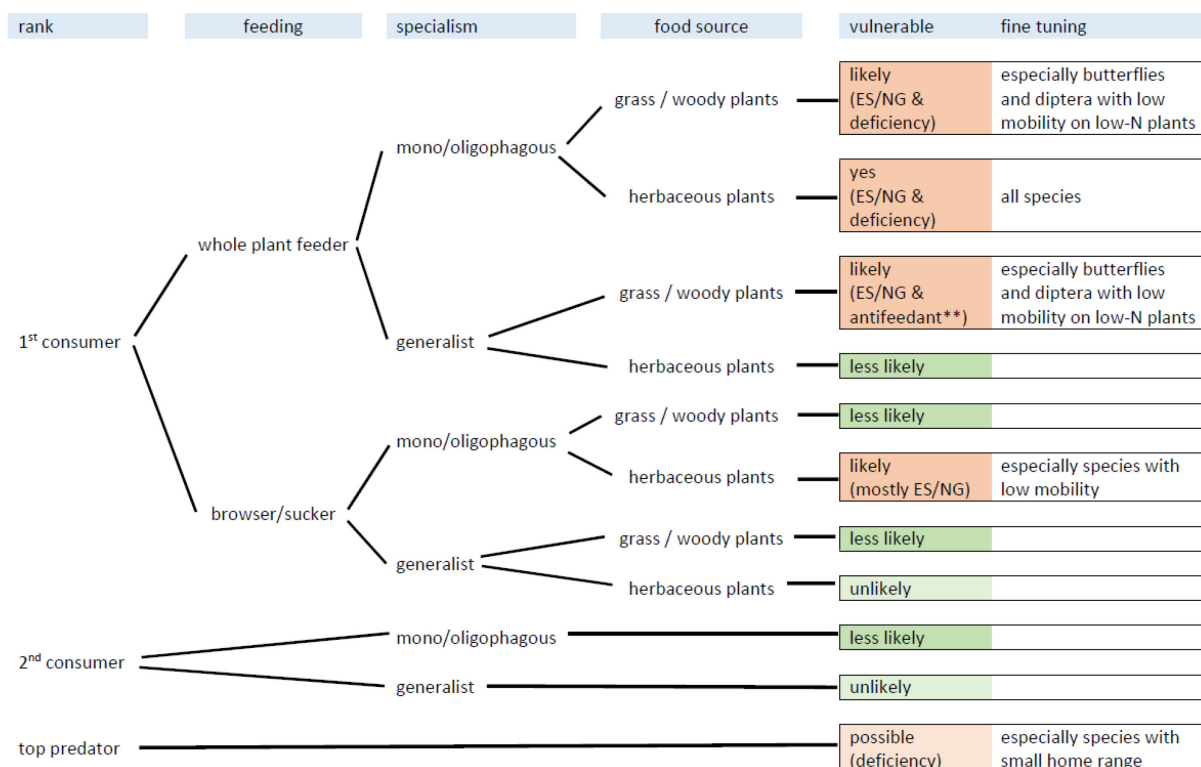
Figure 30. *Simplified decision tree used in the vulnerability assessment of N2000 associated animal species for changes in food quality due to increased N-deposition based on 1) rank in foodweb, 2) feeding behavior, 3) specialist or generalist feeder and 4) food source of 1st order consumers. Vulnerability noted with ES/NG means relevant effects on in C:N:P stoichiometry (Ecological Stoichiometry) or protein:carbohydrate ratios (Nutritional Geometry) are expected; vulnerability noted with 'deficiency' means relevant effects on availability of (essential) micronutrients or (trace) elements are expected; vulnerability noted with 'antifeedants' means significant effects of changed antifeedant concentrations are expected. * Butterflies and dipterans are believed to be more vulnerable due to phylogenetic constraint (see text). **Relevant changes in antifeedant content mostly expected for species feeding on plants naturally low in nitrogen. For (generalist) species feeding on woody plants, increased N-deposition can (temporarily) improve nutritional quality, leading to outbreaks.*

Figuur 30. *Vereenvoudigde beslisboom gebruikt bij de kwetsbaarheidsbeoordeling van aan N2000 habitattypen geassocieerde diersoorten voor veranderingen in voedselkwaliteit als gevolg van verhoogde N-depositie. De beoordeling is gebaseerd op 1) plaats in het voedselweb, 2) voedingsgedrag, 3) voedselspecialist versus generalist en 4) het type voedselbron van 1^e orde consumenten. Kwetsbaarheid aangemerkt met ES/NG betekent relevante effecten verwacht op C:N:P stoichiometrie (ecologische stoichiometrie) of proteïne:koolhydraat ratio's ('nutritional geometry'); kwetsbaarheid aangemerkt met 'deficiëncy' betekent dat relevante effecten worden verwacht op de beschikbaarheid van (essentiële) micronutriënten of (sporen) elementen; kwetsbaarheid aangemerkt met 'antifeedants' betekent dat er significante effecten worden verwacht als gevolg van gewijzigde antifeedant concentraties. *Aangenomen wordt dat vlinders en tweevleugeligen kwetsbaarder zijn vanwege fylogenetische beperking (zie tekst). **Relevante veranderingen in antivraatstoffen wordt verwacht bij soorten die zich voeden met planten die van nature weinig stikstof bevatten. Voor (generalitische) soorten die zich voeden met houtige planten kan een verhoogde N-depositie (tijdelijk) de voedingskwaliteit verbeteren - wat resulteert in plaagvorming.*

8.2 Results

8.2.1 N2000 habitat types

Although simplified, the decision tree (**Figure 29**) provides a standardized and logical analyses of the likely vulnerability for relevant changes in food quality for consumers within different habitat types. It should be realized that changes in food availability caused by a decrease of (preferred) host plants or prey due to changes in soil chemistry and encroachment by tall grasses or shrubs are not included in this analysis. Using this decision tree as a template, we conclude that stable habitats with oligotrophic to mesotrophic substrate (soil or water) with N limitation or N/P co-limitation with low ANC are (probably) always vulnerable to increased N-deposition effects on nutritional quality of producers for consumers. This effect may encompass C:N:P stoichiometric changes, changes in essential (trace) elements, amino acids, fatty acids or (micro)nutrients or allelochemicals. Only producers in naturally N rich, P-limited habitats are unlikely to be vulnerable to increased N-deposition, but these habitats are scarce, especially in terrestrial ecosystems. The responses of plant production to P-limitation can only be understood when interactions with other abiotic resources (like N) and biotic interactions are taken into account (Cramer 2010).

Evidence is growing that plasticity in growth of plants in combination with soil biochemical processes which are altered by the availability of N and P results often in a strictly or primarily co-limitation of N and P. According to a large meta-analysis by Harpole et al. (2011), more than half of the studied aquatic and terrestrial ecosystems are N/P co-limited. When extra N becomes available, habitats do not automatically become P-limited, probably because N fertilization can enhance phosphatase activity, both on plant roots and in soils (Güsewell 2004, Marklein and Houlton 2012). An alternative to the law of the minimum is the multiple limitation hypothesis (MLH) which states that plants adjust their growth patterns such that they are limited by several resources simultaneously, which is supported by the analysis of Ågren et al. (2012).

In this report only habitats where P is tightly bound to the soil complex, such as in calcium phosphate or Iron(III) phosphate, are considered P limited and therefore less likely to be affected by N-deposition: calcareous dune grassland (H2130_A), Buckthorn shrubs (H2160), Transition mires and quaking fens (H7140) and Xeric sand calcareous grasslands (H6120) in river floodplains. The dynamic processes in coastal white dunes (H2120) and inland active drift sands (H2330), mainly erosion and accumulation of sand by water and wind, can decrease overall levels of N and P availability as well as recharge of the ANC which makes effects of increased N-deposition on plant nutritional quality less likely in these habitats. Oligo- to mesotrophic habitats with higher ANC are probably vulnerable for changes in macronutrient stoichiometry, but less so for deficiency of micronutrients. Meso- to eutrophic habitats are less or highly unlikely to be vulnerable for increased N-deposition, especially when also ANC is high and/or dynamic processes ae dominant.

In **Table 10**, Nature 2000 habitat types are shown which are the expected to be vulnerable for changes in plant quality due to increased N deposition as analysed with the decision tree. All dry heathland types (H4030, H2140_B, H2150, H2320), stable non calcareous dune grassland (H2130_B, H2130_C) and drift sands (H2330, H2310), dry acid woodland (H9190), rain fed peat moor (H7110) and soft water lakes (H3110, H3130, H3160) are considered vulnerable. Oligotrophic to mesotrophic habitats with high ANC and/or (partly) fed by exfiltrating groundwater are considered probably vulnerable, including wet heathlands (H2140_A, H4010_A), recovering peat moor (H7120), fens (H7140), species rich grasslands (H6230, H6410), dune slacks (H2190) and buffered dry and wet woodland (H9110, H9120 and 9160_A). Effects of increased N deposition are expected to be most severe in these habitats, making them a high priority when conducting future research.

Oligo- to mesotrophic habitats under influence of aeolian and hydro-morphological dynamic processes, such as 'white dunes' (H2120), inland active drift sands (H6410) and alluvial grasslands (2180_B and 1330_B) are expected to be less vulnerable for increased N-deposition. However, it should be noted that these dynamic habitats will only be less vulnerable as long as the natural dynamic processes are active. When these processes are inhibited or ceased, the habitats will

quickly stabilize and will then become much more vulnerable to encounter negative effects of N-deposition on producer quality than under natural conditions. When these habitats are in a stabilized sub-optimal state, they score equally high on the priority list for future research. Dunes with *Hippophae rhamnoides* (H2160) are noted as unlikely to be vulnerable, since this species actively fixes atmospheric nitrogen with associated actinorhizal nodules.

Table 10. *Natura 2000 Habitat prioritization (high and intermediate (med); last column) for future research based on expected vulnerability for N-deposition mediated changes in producer quality. Vulnerability of habitat types based on the criteria and decision tree shown in Figure 29. Habitats with low priority are only given when confusion with occur with similar habitats which are assessed as high or intermediate priority. Also note that under chronic N deposition above critical loads, changes in internal abiotic processes (such as depletion of base cations), habitat types here prioritized as 'intermediate' or even 'low' priority can exhibit changed producer quality as a result, and are thus not to be regarded as 'not vulnerable'. Hydrology: dry: not influenced by (ground)water; rain: mainly or exclusively fed by rainwater; groundwater: mainly fed by groundwater; gw poor: fed by base poor groundwater; gw rich: fed by base rich groundwater. Dynamics: dynamic nature of the habitat type, or prerequisite for a habitat to be highly vulnerable (i.e. stabilized dune habitats have higher priority than dynamic dune habitats).*

Tabel 10. *Natura 2000 Habitat prioritering (hoog en intermediair (med); laatste kolom) voor toekomstig onderzoek, gebaseerd op verwachte kwetsbaarheid voor N-depositie gestuurde veranderingen in producentkwaliteit. Kwetsbaarheid van habitattypen op basis van de criteria en beslissingsboom in Figuur 29. Habitats met lage prioriteit zijn alleen vermeld wanneer verwarring kan bestaan met vergelijkbare habitats die worden beoordeeld als hoge of intermediaire prioriteit. Bedenk ook dat onder chronische N-depositie boven kritische waarden, veranderingen in interne abiotische processen (zoals uitputting van basische kationen), habitattypen die hier geprioriteerd worden als 'intermediaire' of 'lage' prioriteit, uiteindelijk ook in producentenkwaliteit zullen worden aangetast. Deze habitattypen zijn dus niet zonder meer te beschouwen als 'niet kwetsbaar'. Hydrologie: 'dry': niet beïnvloed door (grond)water; 'rain': voornamelijk of uitsluitend gevoed door regenwater; 'groundwater': voornamelijk gevoed door grondwater; 'gw poor': gevoed door basenarm grondwater; 'gw rich': gevoed door basenrijk grondwater. Dynamics: natuurlijke mate van dynamiek in een habitattype, of voorwaarde voor een habitattype om gevoelig te zijn voor veranderingen in voedselkwaliteit (bijv. gestabiliseerde versus dynamische duinhabitats).*

N2000 code	Type	Dutch Name	Nutrients	Limitation	ANC	Hydrology	Dynamics	Priority
2130_B	dunes	Grijze duinen kalkarm	oligo	N or NP	acid	dry	stable	high
2130_C	dunes	Grijze duinen heischraal	oligo	N or NP	poor	dry	stable	high
2140_B	dunes	Duinheiden met kraaihei droog	oligo	N or NP	acid	dry	stable	high
2150	dunes	Duinheiden met struikhei	oligo	N or NP	acid	dry	stable	high
2310	dunes	Stuifzandheiden met struikhei	oligo	N or NP	acid	dry	stable	high
2320	heathland	Binnenl. kraaiheibegroeiingen	oligo	N or NP	acid	dry	stable	high
3110	softwater	Zeer zwakgebufferde vennen	oligo	N or NP	poor	rain / gw poor	stable	high
3130	softwater	Zwakgebufferde vennen	oligo	N or NP	poor	rain / gw poor	stable	high
3160	softwater	Zure vennen	oligo	N or NP	acid	rain	stable	high
4030	heathland	Droge heiden	oligo	N or NP	acid	dry	stable	high
5130	heathland	Jeneverbesstruwelen	oligo	N or NP	poor	dry	stable	high
7110_A	peatmoor	Actieve hoogvenen	oligo	N or NP	acid	rain	stable	high
7110_B	peatmoor	Heideveentjes actief hoogveen	oligo	N or NP	acid	rain	stable	high

Table 10. *(continued)*

9190	forest	Oude eikenbossen	oligo	N or NP	acid	dry	stable	high
N2000 code	Type	Dutch Name	Nutrients	Limitation	ANC	Hydrology	Dynamics	Priority
91D0	forest	Hoogveenbossen	oligo	N or NP	acid	rain	stable	high
2120	dunes	Witte duinen	oligo	N or NP	poor	dry	dynamic	low
2330	dunes	Zandverstuivingen	oligo	N or NP	acid	dry	variable	high / med
2140_A	dune slacks	Duinheiden met kraaihei vochtig	oligo	N or NP	acid	gw poor	stable	med
2190_C	dune slacks	Vochtige duinvalleien ontkalkt	oligo	N or NP	poor	gw poor	variable	med
4010_A	dune slacks	Vochtige heiden op zandgrond	oligo	N or NP	acid	gw poor	stable	med
4010_B	heathland	Vochtige heiden in laagveen	oligo	N or NP	acid	gw poor	stable	med
6230	grassland	Heischrale graslanden	oligo	N or NP	poor	dry / gw poor	stable	med
6410	grassland	Blauwgraslanden	oligo	N or NP	poor	ground water	stable	med
7120	peatmoor	Herstellende hoogvenen	oligo	N or NP	acid	rain / gw poor	stable	med
7140_A	fens	Trilvenen	oligo	N or NP	poor	gw poor / sw	stable	med
7140_B	fens	Veenmosrietlanden	oligo	N or NP	poor	gw poor / sw	stable	med
2130_A	dunes	Grijze duinen kalkrijk	oligo	P	buffered	dry	stable	low
2160	dunes	Duindoornstruwelen	oligo	P	buffered	dry	variable	low
2190_A	dune slacks	Vochtige duinvalleien open water	oligo	N or NP	buffered	gw rich / rain	variable	med
2190_B	dune slacks	Vochtige duinvalleien kalkrijk	oligo	N or NP	buffered	gw rich	variable	med
2190_D	dune slacks	Vochtige duinvalleien moeras	oligo	N or NP	buffered	gw rich	variable	med
6110	grassland	Pionierbegroeiingen op rotsbodem	oligo	N or NP	buffered	dry	stable	med
7220	fens	Kalktufbronnen	oligo	N or NP	buffered	gw rich	stable	med
7150	fens	Pionierveg. met snavelbiezen	meso	N or NP	poor	gw poor	stable	med
9110	forest	Veldbies-beukenbossen	meso	N or NP	poor	dry	stable	med
9120	forest	Beuken-eikenbossen met hulst	meso	N or NP	poor	dry	stable	med
9160_A	forest	Eiken-haagbeukenbossen	meso	N or NP	poor	dry	stable	med

Natural mesotrophic and eutrophic lakes (H3140, H3150) and open water in marshes (e.g. H7210) are noted as unlikely to be vulnerable for changes in food quality due to increased N deposition. However, a seasonal effect may occur during summer, when in natural conditions these lakes become N limited because most available N is stored in living algal and bacterial biomass and thus has become unavailable for other producers. Increased N-deposition can then mitigate this temporal N limitation, enabling algae to shift in N:P ratio and/or enabling less edible (or toxin producing) algae to become dominant.

8.2.2 N2000 associated species

Although simplified as for habitats, the decision tree provides a standardized and logical means to assess the vulnerability of different species associated with Natura 2000 habitats for N-deposition induced changes in food quality (Figure 30). A list with (probably) vulnerable species is added in Table 11.

Using the decision tree we hypothesize that specialist (mono- to oligophagous) whole plant eaters which feed from herbaceous plants are most vulnerable for effects of N-deposition. Likewise, species specialized on feeding on specific grass or woody species are also vulnerable. This is especially true for butterflies and herbivorous dipterans (due to their low tissue N:P ratio) and for species with a small home range. Within N2000 these are different butterfly species of which caterpillars feed on specific herbs, for instance fritillaries on violets *Viola* sp. and Large copper (*Lycaena dispar*) on Great water dock *Rumex hydrolapathum*. Also butterfly species feeding on low-N grass species, such as Grayling (*Hipparchia semele*) and Tree grayling (*H. statilinus*) are most probably vulnerable for changes in plant quality due to N deposition. The same is true for generalist Short-horned Grasshoppers primarily feeding on low-N grass, like Mottled grasshopper (*Myrmeleotettix maculatus*) and long-horned grasshoppers specialized on feeding on specific (herbaceous) plant parts, which often also require an ample amount of invertebrate prey, like Steppe spiny bush-cricket *(Gampsocleis glabra)* and Saddle-backed bush cricket (*Ephippiger diurnus*).

Generalist species of trees and shrubs, including many moth species are probably also often affected by N deposition mediated changes in plant quality. Within this group also positive effects on species are expected or reported, when N increases in plant biomass and C-based antifeedants decline, leading to outbreak of herbivorous species. However, since dose-effect relations are not linear, these initial outbreak event may gradually change to performance declines with ongoing and/or increasing N-deposition and associated acidification.

For browsers and sap sucking insects, specialists on herbaceous plants are probably vulnerable for increased N-deposition, mainly for changes in C:N:P and/or protein:carbohydrate ratios. These groups include true bugs, aphids and cicadas (Hemiptera), but lack in N2000 regulations. Declines of these (and other non N2000 listed invertebrate groups may however impact insectivorous higher order consumers through altered prey composition or availability.

Finally, invertebrate consumers, but especially (top) predators may encounter deficiencies in specific essential trace elements (for invertebrate consumers Na, K and Mg, for vertebrates mostly Ca in the reproduction period), or in specific amino acids and/or vitamins. Within N2000 this group is until now only identifiable for Ca deficient cases, and therefore only includes species (partly) feeding on vertebrates and calcium rich invertebrates, such as birds of prey, reptiles and Garden dormouse (*Eliomys quercinus*). For insectivorous species, a decrease of abundance and/or diversity of prey species might be expected as indirect effect of N-deposition (Nijssen et al. 2017), but this lays beyond the scope of this assessment.

For other consumer species, mainly generalist browsers and sucking herbivores and 2nd order consumers, effects of changes in food quality due to increased N-deposition are less likely or unlikely to occur, but this does not imply that these effects are proven to be absent. It should be stressed that all estimations made here are merely based on the hypotheses underlying the decision tree. As these are based on the hypotheses obtained from in the other chapters of this report, the underlying mechanisms are often clear - or at least plausible - but substantial evidence for the (causal) effects of increased nitrogen deposition on specific animal species in field situations is in most cases simply not available.

In field situations, N deposition mediated effects on consumers will not only be determined by the vulnerability of the species, but also by the vulnerability of the specific habitat. It is therefore expected that effects on a given species will occur earlier or more pronounced in oligotrophic, poorly buffered habitats than in to mesotrophic, better buffered habitat. This might provide an explanation why many species of open, sandy habitats like fritillaries, grayling, blue-winged grasshopper (*Oedipoda caerulescens*) etc., are performing better or are more common in the more buffered, less acidified coastal dunes compared with the weakly buffered, mineral poor inland heathland and drift sands.

Table 11. Natura 2000 associated species prioritization (high and intermediate; last column) for urgency of future research based on expected vulnerable for N-deposition mediated changes in host plant or prey quality, using the decision tree in **Figure 30**.

Tabel 11. Prioritering van aan Natura 2000 habitats geassocieerde soorten (hoog en intermediair; laatste kolom) voor toekomstig onderzoek, gebaseerd op verwachte kwetsbaarheid voor door N-depositie veroorzaakte veranderingen in waardplant- of prooikwaliteit, op basis van de beslisboom in Figuur 30.

Species	Scientific name	Possible Deficiency	Likely ES/NG and deficiency	Yes ES/NG and deficiency	Priority
Butterflies					
Grizzled Skipper	Pyrgus malvae		x		high
Brown Argus	Aricia agestis			x	high
Dingy Skipper	Erynnis tages			x	high
Niobe Fritillary	Fabriciana niobe			x	high
Little Blue	Cupido minimus			x	high
Purple hairstreak	Favonius quercus		x		high
Small Skipper	Thymelicus sylvestris		x		high
Alcon blue	Phengaris alcon		x		high
Green hairstreak	Callophrys rubi		x		high
Poplar admiral	Limenitis populi			x	high
Dark Green Fritillary	Speyeria aglaja			x	high
Large Copper	Lycaena dispar			x	high
Purple Emperor	Apatura iris			x	high
Silver-studded Blue	Plebejus argus		x		high
Grayling	Hipparchia Semele		x		high
Tree Grayling	Hiparchia statilinus		x		high
White Admiral	Limenitis camilla			x	high
Queen of Spain Fritillary	Issoria lathonia			x	high
Silver-spotted Skipper	Hesperia comma		x		high
Marsh Fritillary	Euphydryas aurinia			x	high
Lesser Marbled Fritillary	Brenthis ino			x	high
Pearly Heath	Coenonympha arcania		x		high
Idas Blue	Plebejus idas		x		high
Cranberry Blue	Agriades optilete		x		high
Cranberry Fritillary	Boloria aquilonaris		x		high
Large Heath	Coenonympha tulia		x		high
Small Pearl-bordered Fritillary	Boloria selene			x	high
Reptiles					
Northern Viper	Vipera berus	x			intermediate
Slow Worm	Anguis fragilis	x			intermediate
Common Lizard	Zootoca vivipara	x			intermediate
Sand Lizard	Lacerta agilis	x			intermediate
Grasshoppers & Crickets					
Blue-winged Grasshopper	Oedipoda caerulescens	x			high
Mottled Grasshopper	Myrmeleotettix maculata		x		high
Large Marsh Grasshopper	Stethophyma grossum		x		high
Grey Bush-cricket	Platycleis albopunctata		x		high
Bog Bush-cricket	Metrioptera brachyptera		x		high
Heath Bush-cricket	Gampsocleis glabra		x		high
Field Cricket	Gryllus campestris		x		high
Wart-biter	Decticus verrucivorus		x		high

Table 11. *(continued)*

Species	Scientific name	Possible Deficiency	Likely ES/NG and deficiency	Yes ES/NG and deficiency	Priority
Saddle-backed Bush-cricket	*Ephippiger diurnus*		x		high
Stripe-winged Grasshopper	*Stenobothrus lineatus*		x		high
Birds					
Tawny Owl	*Strix aluco*	x			intermediate
Little Grebe	*Tachybaptus ruficollis*	x			intermediate
Black-necked Grebe	*Podceps nigricollis*	x			intermediate
Great Grey Shrike	*Lanius excubitor*	x			intermediate
Black-crowned Night Heron	*Nycticorax nycticorax*	x			intermediate
Short-eared Owl	*Asio flammeus*	x			intermediate
European Honey Buzzard	*Pernis apivorus*	x			intermediate
Black Tern	*Chlidonias niger*	x			intermediate
Mammals					
Garden Dormouse	*Eliomys quercinus*	x			intermediate
Total		**14**	**23**	**13**	

Part 4: Nederlandstalige samenvatting en kennisagenda

9 Samenvatting

9.1 Aanleiding en doel van deze literatuurreview

Vanaf het begin van de vorige eeuw is de depositie van atmosferisch reactief stikstof (N), in gereduceerde of geoxideerde vorm, wereldwijd fors toegenomen als gevolg van toenemende industrialisatie en de uitvinding van chemische fixatie van N. Regionale verschillen in depositie van N zijn groot: zwaartepunten liggen in het oosten van de Verenigde Staten, India, China en Zuidoost Azië en Noordwest Europa. Binnen Noordwest Europa behoort Nederland voor decennia tot de landen met hoogste jaarlijkse N-depositie. De ecologische impact van verhoogde N-depositie is in Nederland dan ook uitzonderlijk hoog. Voor het behoud en bescherming van natuurkwaliteit in Nederland is kennis over de ecologische effecten van toegenomen N-depositie van groot belang.

Veel studies naar de effecten van N-depositie op ecosysteem functioneren zijn gericht zich op de impact op bodem- en waterchemie, productiviteit en plantengemeenschappen. De effecten op hogere trofische niveaus richten zich op enerzijds indirecte effecten (bijv. verruiging, veranderingen in microklimaat, afname nestgelegenheid) en anderzijds op chemische veranderingen (veranderingen in plantkwaliteit).

Een groot kennishiaat ligt in de effecten van N-depositie op de chemische samenstelling van producenten en de consequenties daarvan op consumenten. In dit rapport wordt uitvoerig aandacht besteed aan dit specifieke aspect. Dit is uitgevoerd door zo veel mogelijk beschikbare relevante literatuur over dit onderwerp bijeen te brengen.

Om de complexiteit en brede omvang van dit onderwerp het hoofd te kunnen bieden, is het rapport onderverdeeld in vier onderdelen. Deel 1 richt zich hoofdzakelijk op '*nutritional frameworks*' zoals die in de ecologie worden gehanteerd, in volgorde van toenemende complexiteit. Deel 2 richt zich specifiek op het effect van toegenomen N-depositie op het functioneren van ecosystemen, de effecten hiervan op de kwaliteit van producenten en het effect ervan op consumenten. Dit deel sluit af met een eerste bescheiden toets van hypothesen, voortvloeiend uit Deel 1 en 2, door middel van een studie naar populatietrends van dagvlinders in Nederland onder verschillende (historische) N-depositie belasting. Deel 3 richt zich op het identificeren en inschatten van de gevoeligheid van in Nederland voorkomende Natura 2000 habitattypen en geassocieerde diersoorten voor het optreden van veranderingen in voedselkwaliteit als gevolg van N-depositie. Deze inschatting berust op dit moment voor een groot deel op aannames gebaseerd op de eerder beschreven delen 1 en 2 en moet gezien worden als een middel om een prioritering van nader onderzoek naar de effecten van N-depositie op voedselkwaliteit te kunnen maken. Deel 4 bestaat uit deze uitgebreide Nederlandstalige samenvatting en sluit af met een bondige kennisagenda voor toekomstig onderzoek.

9.2 Voedselkwaliteit in ecologie

In de ecologie wordt vanuit verschillende invalshoeken onderzoek gedaan naar variatie in voedselkwaliteit en het effect ervan op het functioneren van consumenten. Deze invalshoeken verschillen sterk in mate van complexiteit, van meest basaal naar meest complex: ecologische stoichiometrie (C:N:P), (sporen)element gehalten, eiwitten en koolhydraten, essentiële micronutriënten en antivraatstoffen en fytotoxinen. Met de toenemende complexiteit neemt de hoeveelheid beschikbare literatuur doorgaans echter af, waardoor allereerst vastgesteld kan worden dat de grootste kennishiaten binnen de complexere definities van voedselkwaliteit bestaan.

9.2.1 Ecologische stoichiometrie

In het onderzoeksveld van de ecologische stoichiometrie (ES) is veel onderzoek uitgevoerd naar de ecologische effecten van verschillen in verhoudingen tussen de belangrijkste biologische elementen C (koolstof), N (stikstof) en P (fosfor) in producenten en (hogere orden) consumenten. Het centrale paradigma is dat niet de absolute beschikbaarheid van één van deze elementen, maar de relatieve beschikbaarheid van deze elementen ten opzichte van de andere bepalend is voor (o.a.) populatiegroeisnelheid, reproductie en/of overleving. Deze is optimaal bij een specifieke ratio, de *Threshold Elemental Ratio* (TER), welke sterk kan verschillen tussen soorten. Voor consumenten is de TER onder meer afhankelijk van fysiologie, fylogenie, ontogenie, gedragsmatige aanpassingen en andere soorteigenschappen zoals de mate van dispersie, en de mate van specialisatie op een bepaald type voedselbron of specifieke waardplant. Producenten hebben doorgaans een hogere mate van plasticiteit in C:N:P stoichiometrie, en worden daarnaast gekenmerkt door een hogere C:N en N:P stoichiometrie dan consumenten.

Veranderingen in producent C:N:P stoichiometrie treden op als gevolg van veranderingen in N- en P-beschikbaarheid. Onder toegenomen N-depositie nemen C:N ratio's doorgaans af, maar tegelijkertijd neemt de N:P ratio toe. Deze verschuivingen leiden tot een grotere kans op het optreden van versterkte P-limitatie, of tot een verschuiving van N- naar P-limitatie in consumenten. De kans en de mate waarin N-depositie tot ecologisch relevante veranderingen in producent-consument stoichiometrische interacties leidt is echter ook sterk afhankelijk van het type ecosysteem (oligotroof vs. eutroof, zwak tot goed gebufferd), de specifieke soort producent en de specifieke soort consument. Voor terrestrische ongewervelden bestaat enig ondersteunend bewijs voor de hypothese dat 1) specialisten een smallere 'stoichiometrische niche' bezetten dan generalisten, wat leidt tot een snellere afname van de fitness in de eerste groep bij veranderingen in CNP stoichiometrie, 2) specialisten doorgaans leven van waardplanten die een CNP stoichiometrie kennen die dicht bij de eigen stoichiometrie ligt, 3) er binnen consumenten aspecten van lichaamsgrootte een rol spelen in de CNP stoichiometrie (met doorgaans lagere C:P en N:P waarden in kleine organismen) en 4) vlinders (Lepidoptera) en vliegen & muggen (Diptera) relatief kwetsbaar zijn voor veranderingen in producent N:P ratio's omdat deze groepen een relatief lage N:P ratio hebben in vergelijking tot andere groepen organismen.

9.2.2 Andere (sporen)elementen

Naast de macro-elementen spelen andere elementen een cruciale rol in het functioneren van producenten en consumenten, onder meer in de werking van enzymen, celprocessen en homeostase. Onder natuurlijke omstandigheden treden verschillen in producent-consument ionen ratio's veelvuldig op, en deze verschillen kunnen door N-depositie worden versterkt (via eutrofiëring en/of verzuringsroutes). Gewervelde organismen onderscheiden zich van andere consumenten door een hogere vraag naar Ca en P voor botvorming. De meest gerapporteerde tekorten zijn voor vogels tekorten in Ca, maar voor herbivore zoogdieren is P in plaats van Ca meestal beperkend, en voor deze groep zijn daarnaast tekorten in Na en sporenelementen als Cu en Se aangetoond. Voor ongewervelden zijn alleen die soorten die Ca in hun exoskelet inbouwen gevoelig voor Ca-tekorten. Dit zijn pissebedden (en andere kreeftachtigen), slakken en miljoenpoten en deze organismen vormen vaak een sleutelrol in de voorziening van Ca in insectivore gewervelden. Voor overige ongewervelden is K en Na beschikbaarheid vaak vastgesteld als limiterend, maar ook voor de concentratie van andere elementen (Ca, Mg, Na, Cu) is een positieve bijdrage gevonden aan de dichtheid van herbivore ongewervelden. Voor soorten die leven van stuifmeel is een tekort van Na in de voedselbron dermate groot dat een aanvullende bron voor die groep gesuggereerd wordt (drinken van modder, urine of plantensap). Voor soorten die leven van nectar (vlinders) is eenzelfde tekort aannemelijk, en voor deze groep is het drinken van modder, urine, plantensap, etc. dan ook algemeen voorkomend.

9.2.3 Eiwitten en koolhydraten

Binnen de groep van eiwitten en koolhydraten (vetzuren, vetten, suikers) is veel onderzoek uitgevoerd binnen het *Nutritional Geometry framework* (NG), welke zich richt op het effect van verschillen in de ratio tussen eiwitten en koolhydraten. Overeenkomstig met ES is het centrale

paradigma in NG dat de verhouding van deze twee groepen van voedingsstoffen bepalend is voor de voedselkwaliteit van producenten, en dat verschillende soorten verschillende '*intake targets*' kennen, en verschillende opname strategieën kennen ten opzichte van die *intake target*. Consumenten zijn in staat om de concentratie van beide nutriënten in hun voedsel te detecteren en vertonen dan ook compensatie (meer voedsel eten) en complementair (voedsel van een contrasterende kwaliteit eten) opname gedrag bij gebrek van (één van) deze nutriënten. Bij een laag gehalte van deze voedingsstoffen wordt de voedselopname sterk verhoogd, bij opname van voedsel met een suboptimale verhouding van beide nutriënten wordt bij voorkeur voedsel met een complementaire verhouding geconsumeerd. Dit mechanisme stelt consumenten in staat om de opname van beide nutriënten ten opzichte van elkaar af te stemmen, maar bij een lagere ratio van andere (elementaire) nutriënten treedt er een risico op van tekorten voor dei andere nutriënten, zeker wanneer het voedsel hoge eiwit en/of koolhydraat gehalten kent. In dat geval wordt er immers minder voedsel geconsumeerd omdat de *intake target* voor eiwit en/of koolhydraat al snel bereikt wordt. De overlevingsstrategie van een soort is tenslotte bepalend voor de mate waarin een consument haar voedselopname reguleert, met een striktere mate van homeostase bij (laag mobiele) specialisten (geselecteerd naar compensatie voedselopname) en een minder sterke mate van homeostase bij (hoog mobiele) generalisten (geselecteerd naar complementaire voedselopname). Onder veranderende eiwit-koolhydraat verhoudingen zullen specialisten derhalve eerder negatieve gevolgen ondervinden dan generalisten (in het geval dat dit leidt tot een eiwit-koolhydraat verhouding die afwijkt van de *intake target* of tot verdunning van andere elementen in het voedsel).

9.2.4 Essentiële micronutriënten

Essentiële micronutriënten zijn in dit rapport gedefinieerd als organische moleculen welke niet of in onvoldoende mate gesynthetiseerd kunnen worden door consumenten, en dus volledig of gedeeltelijk uit het voedsel moeten worden verkregen. Grofweg zijn deze in drie typen te onderscheiden: essentiële aminozuren, vetzuren en vitaminen. In ecologische studies zijn onderzoeken naar micronutriënt tekorten zeer schaars. In producenten zijn veel studies uitgevoerd naar het effect van N-depositie op vrije aminozuren. Vrije aminozuren die relatief rijk aan N zijn, nemen in deze studies toe, ten koste van N-arme aminozuren. Vrije aminozuren zijn slechts een fractie van het totaal aan aminozuren in producenten; voor het totaal aan aminozuren in de plant, waarvan een groot deel in eiwit is vastgelegd, heeft N-depositie een veel minder sterk effect op de verschuiving naar N-rijke aminozuren. Echter, bij relatief zeldzame essentiële aminozuren neemt het absolute gehalte soms sterk af. Hoewel in de procentuele samenstelling van het eiwit het effect gering is (omdat het zeldzame aminozuren betreft), zijn deze verschuivingen toch ecologisch relevant. Voor consumenten zijn effecten van aminozuurtekorten beschreven in veterinaire studies. In eieren van vogels zijn veel embryonale afwijkingen toe te schrijven als een gevolg van aminozuurtekorten, als gevolg van een tekort van 1 of meerdere specifieke aminozuren, of door een ongunstige samenstelling van de aminozuren. In aquatische systemen lijkt een tekort aan aminozuren bij consumenten niet of nauwelijks op te treden. Dit is waarschijnlijk het gevolg van een sterke symbiose tussen zoöplankton en aminozuur producerende micro-organismen, waardoor alle essentiële aminozuren aan de basis van de heterotrofe voedselketen in adequate hoeveelheden beschikbaar zijn.

Essentiële vetzuren die door producenten worden gesynthetiseerd zijn *poly unsaturated fatty acids* (PUFA's) en *highly unsaturated fatty acids* (HUFA's). Van de laatste is *docosahexaenoic acid* (DHA) van groot belang in consumenten. In aquatische systemen is het gehalte van HUFA's in producenten doorgaans veel hoger dan in van terrestrische systemen. HUFA's spelen een belangrijke rol in het behoud van membraan plasticiteit en spelen een rol als precursor molecuul voor signaalstoffen zoals eicosanoïden, die een belangrijke rol spelen in ontstekingsreacties. Een tekort in HUFA's leidt in mensen tot gereduceerde groei, gewichtsverlies, afgenomen gezichtsvermogen en het kan leiden tot het ontwikkelen van tumoren. HUFA's bioaccumuleren hoger in voedselketens. In aquatische systemen hebben consumenten, en in terrestrische systemen de toppredatoren doorgaans niet het vermogen om HUFA's te synthetiseren uit PUFA's.

Voor de soorten die dit vermogen wel hebben is een HUFA-arm dieet echter nog steeds suboptimaal en leidt vaak tot deficiëntie symptomen.

Het gehalte van vitaminen in producenten verandert doorgaans niet direct als gevolg van N-depositie, maar dit kan als gevolg van aminozuurtekorten (vitamine B2) of verzuring en geassocieerde afname van essentiële sporenelementen voor producenten, wel indirect optreden (vitamine B12, A). Eveneens indirect door N-depositie beïnvloed is thiamine (vitamine B1), wat in aquatische systemen kan leiden tot thiamine deficiënties in consumenten. Hier is dit niet gerelateerd aan het vitamine zelf, maar aan de productie van thiaminasen als antivraatstof door aquatische producenten. Thiamine deficientie kan leiden tot sterk verhoogde mortaliteit in vroege ontwikkelingsstadia van vissoorten. Een aantal soorten is echter in staat om de thiaminasen in hun weefsels op te slaan, wat vervolgens kan leiden tot verhoogde sterfte onder predatore consumenten.

9.2.5 Antivraatstoffen en fytotoxinen

Secundaire plantenstoffen hebben vaak een functie in afweer tegen herbivorie. Antivraatstoffen verminderen veelal de voedselkwaliteit door het reduceren van de voedselopnamecapaciteit van consumenten, fytotoxinen zijn direct giftig voor consumenten. Deze stoffen kunnen koolstofverbindingen, of een combinatie van koolstof- en stikstofverbindingen zijn. Bij een verlichting van N limitatie voor planten kunnen N gebaseerde secundaire metabolieten in concentratie toenemen. Andersom nemen C gebaseerde stoffen vaak (maar niet altijd) af onder een verlichting van N limitatie. Het optreden van droogtestress is vaak een tweede voorwaarde voor een afname van C gebaseerde antivraatstoffen onder toegenomen N last. Planten die specifieke fytotoxinen produceren om vraat zo veel mogelijk te beperkten hebben vaak gecoëvolueerde specialistische herbivoren welke in staat zijn om deze gifstoffen te neutraliseren of op te slaan in speciale opslagstructuren in het lichaam. Deze soorten zijn daardoor weinig gevoelig voor veranderingen (toe of afname) van antivraat- of gifstoffen. Generalistische soorten hebben een grotere gevoeligheid voor veranderingen in concentraties van antivraatstoffen en/of fytotoxinen. Onder invloed van N-depositie zullen generalistische soorten sterker reageren op veranderingen in concentraties secundaire metabolieten. Dit betekent dat bij planten met een C gebaseerde afweer, die groeien onder van nature N gelimiteerde omstandigheden, vaak een sterkere vraat van generalistische soorten kan optreden onder toegenomen N-depositie. Andersom kan een toename van N gebaseerde antivraatstoffen leiden tot een afname van de fitness van generalisten, maar dit zal in de praktijk zelden optreden, aangezien de meeste planten geen tot weinig gebruik maken van N gebaseerde secundaire plantenstoffen in hun afweer.

9.2.6 Adaptaties tegen lage of ongebalanceerde voedselkwaliteit

Consumenten hebben gedurende de evolutie een breed scala aan aanpassingen ontwikkeld om met onvoldoende voedselkwaliteit om te gaan. In de hierboven beschreven paragrafen is bijvoorbeeld vaak een verschil tussen voedsel en/of waardplant specialisten en generalisten genoemd, welke kan gezien worden als twee contrasterende sets van specifieke aanpassingen, beide (ten dele) gericht op het verkrijgen van een adequate hoeveelheid voedselopname, maar op twee sterk verschillende wijzen. Predatie en omnivorie kunnen ook beschouwd worden als verschillende voedselstrategieën gericht op het eenvoudiger maken van een adequate voedselopname, tegen kosten van verhoogde risico's met betrekking tot mortaliteit en/of voedselschaarste, en hebben vaak consequenties voor de gevoeligheid voor specifieke tekorten (een goed voorbeeld is een verschil in gevoeligheid tussen toppredatoren en primaire consumenten in hun gevoeligheid voor micronutriënten tekorten).

Specifieke aanpassingen zijn dus vaak facultatief of obligaat verbonden met andere aanpassingen, afhankelijk van de voedingsniche van de soort. De gehele set van aanpassingen vormt de *Life-history tactic* (LHT) van het organisme, welke uiteindelijk de gevoeligheid van een organisme voor veranderde voedingskwaliteit bepaalt. Adaptaties kunnen fysiologisch van aard zijn, zoals het verhogen van de affiniteit of synthese van enzymen die faciliteren in de opname van limiterende nutriënten, het kunnen gebruiken van meerder precursor moleculen in de synthese van

micronutriënten of het opslaan van essentiële voedingsstoffen in lever of vetweefsel. Een andere belangrijke aanpassing is symbiose met micro-organismen die consumenten in staat stelt om aan voedingsstoffen te komen die in het voedsel in onvoldoende hoeveelheid aanwezig is. Gedragsaanpassingen bij inadequate voedselopname is een derde belangrijke aanpassing en omvat onder meer het in NG uitgewerkte compensatie en complementair opname gedrag, maar ook het eten van 'vreemde' stoffen zoals modder, urine of dierlijke botten is gericht op het verminderen of opheffen van nutriënt tekorten. Onder koudbloedigen is thermoregulatie een aanvullende strategie om een overtollige hoeveelheid koolhydraten 'weg te kunnen werken', aangezien de respiratiesnelheid toeneemt bij hogere temperaturen.

In studies gericht op N-depositie-effecten op specifieke soorten consumenten of de samenstelling van soortgemeenschappen van consumenten is voldoende kennis over de LHT die de soort toepast om voldoende voedingsstoffen binnen te krijgen cruciaal om hypothesen op te stellen en/of de resultaten van N-manipulatie-experimenten te kunnen interpreteren. Het gebruik van soorteigenschappen of LHT gebaseerde benadering in veldstudies kan dus een mechanistisch inzicht verschaffen in de waargenomen effecten in de samenstelling van de gemeenschap en verklaren waarom contrasterende responsen van soorten of soortengroepen op verhoogde N-depositie optreden.

9.3 Effecten van N-depositie op voedselkwaliteit

9.3.1 Effect op abiotische processen in bodem en water

N-depositie leidt tot eutrofiëring van natuurlijke meso tot oligotrofe ecosystemen in zowel terrestrische als aquatische milieus. Bovendien kan N-depositie in aquatische systemen ook significante ecologische implicaties hebben in relatief eutrofe wateren wanneer stikstof tijdelijk beperkend raakt, wat kan optreden in de zomer wanneer de nitrificatiesnelheid hoog is.

N-beschikbaarheid in bodem en water neemt geleidelijk toe met een hogere N-belasting vanuit de atmosfeer, waardoor uiteindelijk de C:N-verhouding van de bodem aanzienlijk afneemt. Tegelijkertijd neemt de N:P-verhouding van bodem en water toe. Deze veranderingen in de relatieve beschikbaarheid van elementen kunnen leiden tot veranderingen in de chemie van producenten.

Als gevolg van toegenomen N-depositie neemt de snelheid van verzuringsprocessen toe. Dit leidt tot verlies van zuur bufferend vermogen, een lagere bodem-pH, verhoogde uitspoeling van basische kationen (Ca^{2+}, K^+, Mg^{2+}) en verhoogde concentraties van potentieel giftige metalen (o.a. Al^{3+}). Bovendien leidt verzuring tot een afname van de nitrificatiesnelheid - waardoor de concentratie van NH_4^+ ten opzichte van NO_3^- toeneemt. Deze verzuringseffecten beïnvloeden de plantenchemie aanzienlijk.

9.3.2 Effecten op producenten en consumenten

9.3.2.1 C:N:P stoichiometrie

Als gevolg van door N-depositie veroorzaakte veranderingen in de bodem- en waterchemie kan de chemische samenstelling van producenten ook aanzienlijk veranderen. Vooral onder oligotrofe, zwak gebufferde of zure omstandigheden neemt de concentratie van N in producenten toe zonder een gelijktijdige toename van het C- of P-gehalte, en er dus verschuivingen in de C:N:P ratio's optreden. Deze verhoogde opname van N gaat vaak gepaard met een toename van N-rijke vrije aminozuren, vooral wanneer NH_4^+ de dominante vorm van beschikbaar N is. Meta-analyses rapporteren een significante afname van de C:N ratio en een gelijktijdige toename van N:P ratio onder toegenomen N-depositie. De respons van consumenten op door N-depositie veroorzaakte veranderingen in elementaire stoichiometrie is echter niet eenduidig positief of negatief. Er zijn zowel studies die een toename van fitness of vraat op een verlaagde C:N-verhouding rapporteren als een afname van fitness, waarbij de afname meestal in verband wordt gebracht met een toegenomen N:P ratio. Deze contrasterende reacties van consumenten kunnen worden toegeschreven aan 1) verschillen in C: N: P stoichiometrie tussen studies (een verlaging van de

C:N ratio zonder veranderingen in de N:P ratio zal eerder leiden tot een toename van de fitness in tegenstelling tot een lagere C:N maar tegelijkertijd verhoogde producent N:P ratio); 2) verschillen in de soort specifieke TER van consumenten en 3) de mate van specialisatie op voedselplanten van consumenten. De reactie van consumenten op veranderde C:N:P stoichiometrie is afhankelijk van de specifieke TER, welke op haar beurt afhankelijk is van fysiologische eigenschappen en/of LHT van desbetreffende consument. Door N-depositie geïnduceerde veranderingen in producent C:N:P verhouding kunnen dus leiden tot een toename of afname van individuele soorten, wat in het geval van een nivellering van deze verhouding in de vegetatie leidt tot een afname van diversiteit en/of soortenrijkdom van de gemeenschap van consumenten. De hoeveelheid N-depositie gerelateerde studies naar CNP stoichiometrie effecten op consumenten is tot op heden nog vrij beperkt, met name in het terrestrisch milieu.

9.3.2.2 Overige (sporen)elementen

Parallel aan veranderde verhoudingen in de belangrijkste elementen C, N en P, is herhaaldelijk aangetoond dat toegenomen N-depositie significante implicaties heeft voor N:element verhoudingen. Onder toegenomen N-depositie worden hogere N:K, N:Mg en vaak ook N:Ca verhoudingen gemeten, wat betekent dat de concentratie van deze elementen effectief wordt verlaagd. Voor sporenelementen is minder gerapporteerd, maar ook voor deze elementen worden verdunningseffecten door toegenomen N-depositie gemeld. Het precieze werkingsmechanisme (uitspoeling versus verdunning door groei), de mate waarin dit optreedt en het specifieke element dat beperkend wordt is echter sterk afhankelijk van specifieke ecosysteemeigenschappen zoals bodem- en watertype, bufferstatus, temperatuur, vocht, etc. het optreden van (sporen)element tekorten moet dus worden onderzocht in een ecosysteem-specifieke context.

Binnen de context van toegenomen N-depositie is Ca-deficiëntie bij gewervelde insectivoren het vaakst gemeld, en dan als gevolg van versnelde bodemverzuring. Het mechanisme achter verminderde Ca-beschikbaarheid is echter niet direct gerelateerd aan een afgenomen Ca-beschikbaarheid in prooidieren, maar door de toegenomen zuurgraad van de bodem, waarin prooisoorten met een Ca-rijk exoskelet steeds schaarser worden. In aquatische systemen is er enig bewijs dat andere elementen (Fe, Zn) beperkend kunnen worden voor consumenten, wat ook is aangetoond in terrestrische consumenten voor Na, Mg, K en sporenelementen als Cu en Co. Tot nu toe zijn er echter geen studies uitgevoerd die de door N-depositie veroorzaakte vermindering van de beschikbaarheid van deze elementen (anders dan Ca en Co) bij producenten in verband brengen met verminderde fitness van consumenten.

9.3.2.3 Eiwitten en koolhydraten

Ook voor eiwitten en koolhydraten bestaat er een discrepantie tussen de hoeveelheid publicaties over voedingsexperimenten (*Nutritional Geometry*) en specifieke studies hiernaar in een N-depositie context. In N-depositie effect studies wordt meestal de C:N ratio van de producent als proxy gebruikt voor de verhouding tussen koolhydraten en eiwitten. In veel gevallen is deze versimpeling waarschijnlijk onterecht, aangezien een toegenomen N-depositie ook invloed heeft op de verhouding van vrije aminozuren en N-rijk opslageiwit in plaats van functioneel eiwit bij producenten. Binnen het *NG framework* wordt verondersteld dat een algehele toename van het eiwitgehalte in producenten bij consumenten tot gevolg heeft dat een verminderde totale opname van koolhydraten zal optreden (mogelijk ook van andere voedingsstoffen, zoals P, K, Mg). Daarnaast zijn er studies die onder een verhoogde N-depositie het eiwitgehalte bij producenten niet zien toenemen ondanks een verhoogde totaal-N concentratie, hetgeen nogmaals benadrukt dat het beschouwen van de C:N-verhouding als ruwe maat voor het eiwitgehalte slecht bruikbaar is om interacties tussen producenten en consumenten in veldsituaties te onderzoeken. Geconcludeerd wordt dat er een kennislacune bestaat in de effecten van N-depositie op de beschikbaarheid van eiwitten en koolhydraten in producenten en het effect daarvan op de fitness van consumenten.

9.3.2.4 Micronutriënten

Eiwitten zijn opgebouwd uit aminozuren en een toename van N-depositie kan ingrijpende effecten hebben op de aminozuursamenstelling van producenten. In het geval door opnameregulatie het

totale geconsumeerde 'ruwe eiwit' de totale voedselopname bij consumenten reguleert, kan een toename van opslageiwit in producenten een tekort aan essentiële aminozuren in de consument veroorzaken, in het geval deze minder (of niet) worden gebruikt in opslageiwit. Dit effect kan sterke invloed uitoefenen op consumenten die hun eiwitopname strikt reguleren. Tekorten aan essentiële vetzuren kunnen ook eerder ontstaan bij een verhoogd proteïnegehalte van producenten onder strikte regulering van de eiwitopname. Opname regulatie mechanismen zijn dus van belang om de effecten van door N-depositie veranderde totale koolhydraat:eiwitverhoudingen te kunnen duiden, maar ook die van essentiële koolhydraten en aminozuren van producenten op consumenten. De weinige op dit aspect gerichte terrestrische en aquatische studies geven aan dat verhoogde N-depositie eerste en hogere orden consumenten negatief kan beïnvloeden via veranderde samenstelling van aminozuren, essentiële vetzuren en/of essentiële vitaminen in producenten. Ook hier bestaat nog een grote kennislacune over de mate waarin dit optreedt en wat de exacte werkingsmechanismen zijn.

9.3.2.5 Antivraatstoffen

N-depositie kan de samenstelling en concentratie van secundaire plantenstoffen bij producenten beïnvloeden. Vooral onder van nature oligotrofe omstandigheden nemen concentraties N gebaseerde metabolieten toe en die puur op C gebaseerde af, wanneer het N aanbod experimenteel wordt verhoogd. Of deze gangbare regel ook opgaat onder chronische N-depositie is echter twijfelachtig, aangezien groei van producenten uiteindelijk niet langer N gelimiteerd is en uiteindelijk C allocatie aan groei niet verder meer toe neemt (met als gevolg weer een hogere allocatie van C aan secundaire metabolieten). Wanneer toegenomen N beschikbaarheid leidt tot verlaagde gehalten van C gebaseerde metabolieten neemt de voedselkwaliteit voor herbivoren toe, met name voor slecht aan antivraatstoffen en fytotoxinen aangepaste generalistische herbivoren. Dit is, in samenhang met een eventuele opheffing van N gelimiteerde groei een mogelijke verklaring voor het optreden van insectenplagen in arme bossen en dwergstruikgemeenschappen. Een verhoging van N gebaseerde metabolieten onder verhoogde N-depositie zou echter het tegenovergestelde effect hebben, vooral voor generalistische consumenten, van nature oligotrofe ecosystemen zijn maar weinig producenten die een N gebaseerde verdedigingsstrategie hanteren (met uitzondering van de Leguminosae). De exacte uitkomst van N-depositie op allelochemische interacties tussen producenten en consumenten is dus afhankelijk van de specifieke verdedigingsstrategie van de producent, de specifieke aanpassingen en reguleringsmechanismen van de consument voor antivraatstoffen en fytotoxinen, welke verder gemoduleerd wordt door mogelijke veranderdingen in nutriëntgehalten van producenten. Ook hier staat het ontbreken van voldoende N-depositie gerelateerde case studies het niet toe om algemeen geldende conclusies te trekken.

9.3.3 Hogere trofische niveaus

Hoewel opname-regulering voor C:N:P en/of eiwitten en koolhydraten ook bestaat bij predatoren, zullen N-depositie-effecten op de kwaliteit van prooien waarschijnlijk sterk worden gedempt door opnameregulatie van deze prooidieren. Daarom wordt verwacht dat hogere trofische niveaus veel minder gevoelig zijn voor door N-depositie veroorzaakte veranderingen in producent kwaliteit. Dit met uitzondering van zeldzame essentiële aminozuren die onder invloed van stikstofdepositie minder worden aangemaakt door producenten en vaak slecht accumuleren hoger in de voedselketen. Vaker is te verwachten dat hogere trofische niveaus door indirecte, door N-depositie veroorzaakte veranderingen in prooidiversiteit en -aanbod beïnvloed worden.

De schaarse studies die N-depositie effecten op hogere orde consumenten hebben onderzocht indiceren evenwel dat de gevolgen van deze veranderingen in prooiaanbod aanzienlijk kan zijn. Voor insectivore vertebraten (met name vogels) is vaak melding gemaakt van door verzuring veroorzaakte vermindering van de beschikbaarheid van Ca (door reductie van Ca-rijke prooien) wat individuele populaties en totale vogelgemeenschappen sterk kan beïnvloeden. Consumenten van hogere orde kunnen dus worden beïnvloed door N-depositie via verminderde prooibeschikbaarheid en/of -variatie, waardoor een verminderde opname van essentiële voedingsstoffen optreedt.

Dergelijke mechanismen zijn vooralsnog vooral voor Ca aangetoond, maar kunnen evengoed voor andere (micro)nutriënten optreden.

9.3.4 Dagvlinder trendanalyse

Door middel van een trendanalyse van een set vooraf geselecteerde soorten dagvlinders zijn hypothesen geformuleerd op basis van de hoofdstukken 2 en 3 op geldigheid getoetst, gezamenlijk geformuleerd als volgt: "Waardplantspecialisten van oligotrofe habitats zullen eerder negatieve effecten van veranderde voedselkwaliteit ondervinden dan waardplantgeneralisten van meso- tot eutrofe habitats". Hiervoor zijn lange-termijn populatietrends van dagvlinders uitgevoerd in gebieden die verschillen in (historische) jaarlijkse stikstofdepositie. De resultaten ondersteunen de hypothese: de richting van lange termijn trends kon meestal worden voorspeld op basis van een combinatie van de "voedingsniche" van een soort in combinatie van waardplantspecificiteit en de hoogte van N-depositie in het leefgebied, hoewel er ook uitzonderingen bestonden, en voor een aantal soorten de dataset in hoog depositie gebieden zeer klein was. Het opnemen van meer populatiegegevens, toevoegen van relevante soortenkenmerken (mobiliteit, temperatuurniches, enz.) en/of het gebruiken van een meer gedetailleerde dataset met betrekking tot de kenmerken van waardplanten, N-depositie en bodemchemie zijn aan te bevelen om deze LHT hypothese nader te toetsen.

9.4 Algemene conclusie en hypothesen

Allereerst kan geconcludeerd dat het aantal specifieke aan N-depositie gerelateerde studies naar de relatie tussen producenten en consumenten te laag is om voor de in dit rapport uitgewerkte voedselkwaliteitsaspecten verstrekkende conclusies te trekken over algemeen geldende mechanismen, richting en/of de effectgrootte van N-depositie op de producent-consument relaties. Over het algemeen neemt de hoeveelheid studies af met de mate van complexiteit van het specifieke toegepaste voedselkwaliteitsaspect ("*nutritional framework"*), maar zelfs in het meest eenvoudige framework (ecologische stoichiometrie) bestaan kennishiaten in de werkingsmechanismen en de invloed van omgevingsfactoren en soorteigenschappen op het effect van N-depositie op de fitness van consumenten.

Los van de constatering van grote kennisgaten in N-depositie specifieke studies, zijn op basis van de in de literatuur beschreven mechanismen in meer algemenere zin (beschreven in de review van de "*nutritional frameworks"*) wel uitspraken te doen over de belangrijkste en/of waarschijnlijke causale verbanden tussen toegenomen N-depositie en de effecten daarvan op plantkwaliteit.

Soorten die relatief nauwe niches in meso- tot oligotrofe, slecht gebufferde tot zure habitats bezetten lopen de grootste kans om negatieve effecten van door N-depositie veranderde voedselkwaliteit te ondervinden. Veranderde voedselkwaliteit onder chronisch verhoogde N-depositie vormt voor deze groep een extra bedreiging voor een duurzaam behoud van populaties. Voor N2000 beschermde habitats en daaraan geassocieerde soorten heeft deze conclusie grote implicaties, aangezien veel van de soorten die als kenmerkend gelden voor oligotrofe en/of slecht gebufferde N2000-habitats aan deze soortomschrijving voldoen. Kennisverbreding en verdieping over de exacte werkingsmechanismen en de ernst van de impact van N-depositie op voedselkwaliteit is nodig om een adequaat N emissie reductiebeleid vorm te geven en om maatregelen te ontwerpen die deze nadelige effecten kunnen mitigeren.

Op basis van de mechanismen en hypothesen die in hoofdstuk 2 zijn geformuleerd en de bestaande kennis die in hoofdstuk 5 is samengevat, kunnen verschillende toetsbare hypothesen en richtingen voor toekomstig onderzoek worden geformuleerd. In volgorde van toenemende complexiteit (niet in prioriteit) zijn dit:

- Of een consument profiteert of last ondervindt van verhoogde N-depositie, hangt af van het effect van N-depositie op de stoichiometrische C:N:P en/of C:element en N:element verhouding van producenten, en de richting van deze verandering in relatie tot de soort

specifieke *Threshold Elemenal Ratio* (TER) of *intake target* voor nutriëntopname (zie de paragrafen 2.2.2; 2.4.3; 3.1; 3.3; 5.2 en 5.4).

- De algehele soortenrijkdom van consumenten zal onder verhoogde N-depositie verminderen: een beperkte set van soorten zal een toename in fitness ondergaan, terwijl een mogelijk grotere set van soorten een afname van fitness zal laten zien. Dit proces is mechanistisch verklaarbaar door de soortspecifieke TER en/of intake target van soorten consumenten (2.2.2; 3.1; 5.2 en 5.7).

- Soorten die in sterke mate zijn aangepast aan één of enkele verwante soorten producenten als voedselbron, hebben het grootste risico om een fitness afname te ondergaan bij verhoogde N-depositie. Dit wordt mechanistisch verklaard door een smallere optimale C:N:P bandbreedte voor groei en/of sterkere opnameregulering voor eiwitten en koolhydraten (zie de paragrafen 2.2.2; 2.4.3; 3.1; 3.3; 5.2 en hoofdstuk 6).

- Contrasterend hiermee hebben soorten die een sterk generalistische consumptiestrategie hebben de grootste kans om in fitness toe te nemen als gevolg van verhoogde N-depositie, als gevolg van een bredere optimale C:N:P-bandbreedte, en eventuele verlichting van fitnessafnamen door een verlaagd gehalte aan antivraatstoffen in planten (zie de paragrafen 2.2.2 , 2.4.3, 2.6, 3.1, 3.3, 3.5, 5.2 en hoofdstuk 6).

- De beschikbaarheid van plant Na, Mg, K en sporenelementen zoals Cu, Se en Co kunnen van grote invloed zijn op populaties herbivoren. Uitloging van deze elementen als gevolg van door N-depositie veroorzaakte verzuring kan de populaties herbivoren dus in sterke mate beïnvloeden. Onderzoek hier naar is echter nog schaars en verdient verdere verdieping (zie de paragrafen 2.3, 3.2 en 5.3).

- In N-depositie gerelateerde studies is C:N-ratio een vaak gebruikte maat voor voedselkwaliteit. Op basis van onze studie concluderen wij dat dit een ongeschikte kwaliteitsmaat is. In N-depositie gerelateerde studies verdient het aanbeveling om het effect van N-depositie te kwantificeren op basis van het gehalte aan eiwit en koolhydraat en de specifieke samenstelling ervan (zie de paragrafen 2.4, 3.3 en 5.4).

- Het effect van N-depositie op specifieke eiwit- en koolhydraatgehalten wordt verder gecompliceerd door de invloed er van op de specifieke samenstelling van essentiële aminozuren en vetzuren. Vooralsnog is de samenstelling van aminozuren en vetzuren ondervertegenwoordigd in ecologische producent-consument studies. Omdat er een hoge mate van variatie bestaat in soortspecifieke vereisten wat betreft aminozuren en vetzuren, is voldoende kennis vooraf hierover een voorwaarde om toetsbare hypothesen op te stellen over het effect van N-depositie hierop (zie de paragrafen 2.5, 2.7, 3.5, 3.6, 5.4 en 5.6).

- Het effect van N-depositie op voedselkwaliteit is niet alleen het gevolg van bottom-up effecten via bodem- en producentkwaliteit, ook van de (evolutionaire) aanpassingen van soorten consumenten om onvoldoende voedselkwaliteit het hoofd te bieden (samengevat in paragraaf 2.7). Het gebruik van een soorteigenschappen of een *Life History Tactic* benadering is een belangrijk hulpmiddel bij het testen van de hier geformuleerde hypothesen.

10 Kennisagenda

Om passende beheermaatregelen te kunnen ontwikkelen voor het herstel van voedselkwaliteit (of die rekenschap geven van het behoud van voedselkwaliteit) is meer kennis nodig over de werkingsmechanismen van toegenomen N-depositie op voedselkwaliteit. De basis voor de kennisagenda volgt uit de in hoofdstuk 3 en 7 opgestelde hypothesen en vastgestelde kennislacunes. De wijze waarop deze hypothesen en kennislacunes kunnen worden getoetst en ingevuld is echter afhankelijk van de te beantwoorden vragen. In deze paragraaf stellen we een beknopte werkwijze voor om deze vragen adequaat te kunnen beantwoorden. Deze richt zich op twee **onderzoeksvelden** (procesbegrip en beheer) en bestaat uit drie afzonderlijke **onderzoekslijnen**.

10.1 Procesbegrip

Een belangrijke kennisvraag die op basis van dit preadvies naar voren is gekomen is hoe groot het relatieve belang is van voedselkwaliteitsveranderingen als gevolg van N-depositie. Als we de figuur uit Nijssen et al. (2017) (**Figure 3** in dit rapport) opnieuw beschouwen, heeft dit rapport een belangrijke verdieping opgeleverd. De in deze figuur gehanteerde containerterm "voedselkwaliteit" is hier verder uitgewerkt, en dit preadvies heeft (gedeeltelijk) ook oorzakelijke verbanden kunnen leggen tussen N-depositie enerzijds en verandering van voedselkwaliteit anderzijds. Echter, een belangrijke constatering van dit rapport is ook dat in veel gevallen er onvoldoende bewijs of kennis voorhanden is of, en zo ja, hoe deze kwaliteitsparameters veranderen als gevolg van N depositie.

Terug grijpend op deze figuur, (**Figure 31**) betekent dit allereerst dat er 1) op dit moment nog geen afzonderlijk gewicht gegeven kan worden aan het relatieve belang van de verschillende routes (a-i) waarop N-depositie ingrijpt op faunagemeenschappen. Ook is 2) onduidelijk hoe groot de relatieve bijdrage is van de verschillende in dit rapport behandelde voedselkwaliteitsaspecten (CNP stoichiometrie, (sporen)elementen, macronutriënten, micronutriënten, antivraatstoffen) in veranderingen in voedselkwaliteit. Ten tweede is de uitdaging om meer inzicht te verkrijgen in het werkingsmechanisme; met andere woorden, of, en zo ja, hoe voedselkwaliteit uiteindelijk door toegenomen N-depositie wordt beïnvloed. Kennis over 3) de causale routes van bodem tot (hogere orde) consument is nodig om hier hardere uitspraken over te kunnen doen, en dit betekent dat op meerdere niveaus in die causale route belangrijke kennisvragen moeten worden beantwoord. Deze routes zijn vaak afhankelijk van het type ecosysteem en type voedselweb en waarschijnlijk niet breed geldend, maar de mechanismen (bijvoorbeeld verzuring en bijgevolg uitspoeling van kationen) zijn dat vaak wel.

Tenslotte is een niet onbelangrijke kennisvraag hoe 4) veranderende voedselkwaliteit interacteert met de overige door N-depositie gestuurde veranderingen (d.w.z. anders dan die op voedselkwaliteit). Deze laatste vraag kan nog worden uitgebreid naar andere door menselijk ingrijpen veroorzaakte veranderingen in omgevingsfactoren, zoals veranderingen in vochthuishouding, klimaatverandering en versnippering.

Een **eerste onderzoekslijn** zoekt dus allereerst naar **de belangrijkste voor voedselkwaliteit belangrijke plant chemische processen die door N-depositie veranderen**, en niet meteen naar beheeroplossingen. In het geval van een laag huidig kennisniveau (bijvoorbeeld het relatieve belang van sporenelementen en micronutriënten) kan dit via **exploratief en correlatief onderzoek** worden vergroot, of kunnen experimenteel toetsbare hypothesen worden opgesteld en aangescherpt. Hierbij valt te denken aan veldonderzoek waarbij gradiënten in (historisch-cumulatieve) N-depositie en/of verzuringsgraad worden onderzocht. Hierbij dienen sleutelfactoren met betrekking tot voedselkwaliteit te worden gemeten, zoals bodem- en plantkwaliteit

(samenstelling micro- en macronutriënten), maar ook biodiversiteit, abundantie en/of populatieontwikkeling van consumenten. Om voldoende variatie in N-depositie te kunnen omvatten is dit type onderzoek veelal **internationaal** van aard, omdat de N-depositie op nationaal niveau veelal (te) hoog is.
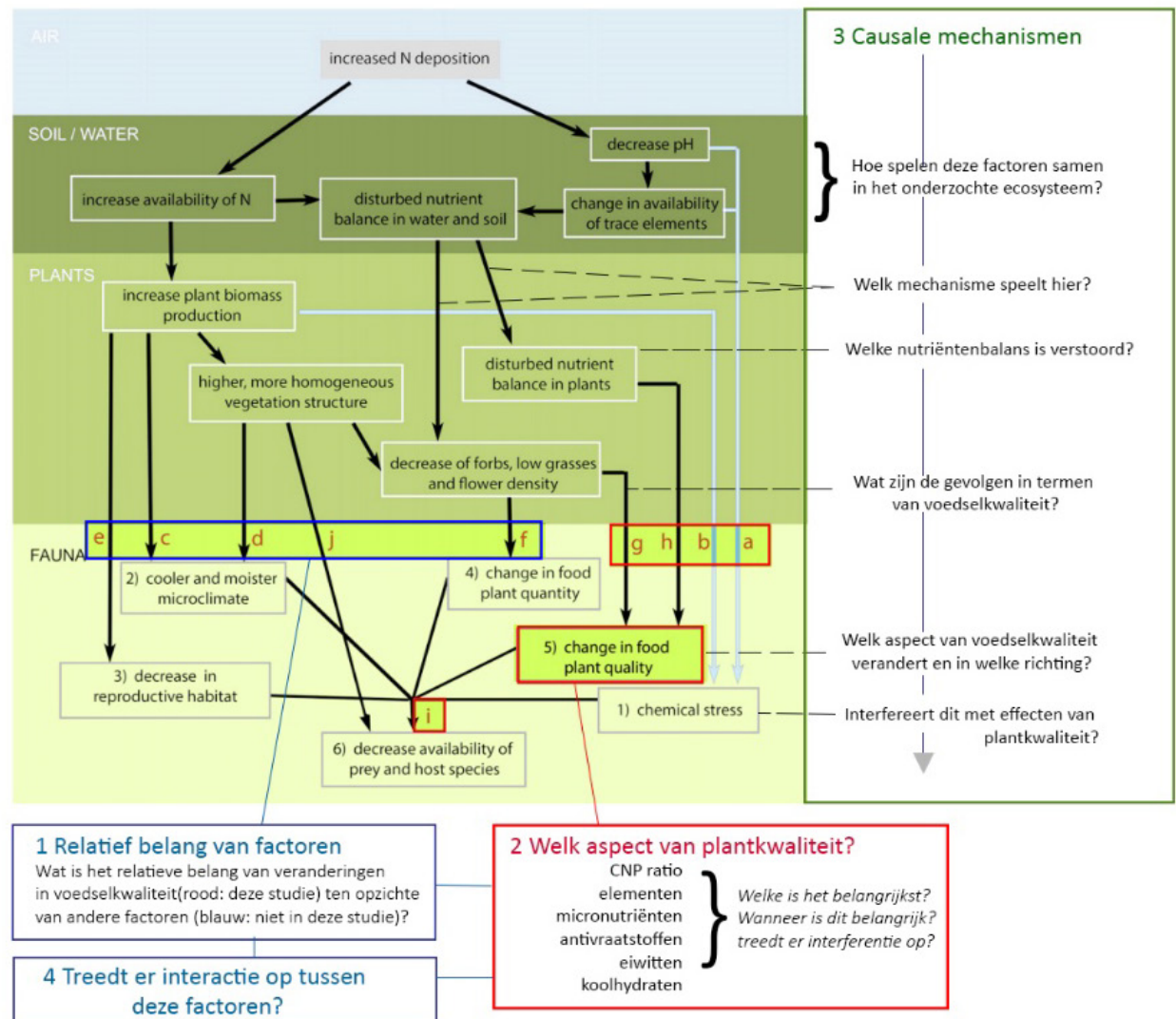
Figuur 31*. De vier belangrijkste thema's die aan bod komen in toekomstig onderzoek naar het effect van N-depositie op voedselkwaliteit.*

Figure 31*. The four main themes addressed for future research into effects of N deposition on food quality.*

Een kansrijke aanpak is door in bestaande, Europa-brede N-bemestingsexperimenten, metingen te verrichten aan plant chemische componenten en aan abundantie, soortenrijkdom of chemie van consumenten in de verschillende behandelplots (N vs. controle). Voor deze studies ligt de focus op **zure tot zwak gebufferde**, **oligotrofe** ecosystemen die van nature **N-, N/P of P-gelimiteerd zijn** en van nature, of door toegenomen vegetatiegroei weinig dynamisch zijn. Binnen het aquatisch milieu zijn wateren die weinig of niet door grond- en/of oppervlaktewater worden gevoed de meest belangrijke systemen, aangezien voor deze systemen de invloed van nitraat en andere vormen van verrijking uit deze niet-atmosferische bronnen gering tot afwezig is.

Een **tweede onderzoekslijn** is die van door N-depositie veranderde producent-consument interacties. Om hypothesen over de verschillende voedselkwaliteitsaspecten te toetsen is **experimenteel onderzoek** nodig. Dergelijk onderzoek wordt gedaan onder sterk **gecontroleerde omstandigheden** (kweekexperimenten). Door slimme keuze van de producent

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(waardplant, alg, cyanobacterie) en consument (specialist/generalist feeder, hoog/laag mobiel, browser/sucker/ grazer, klein/groot, etc.) kan belangrijk inzicht worden verschaft in de onderliggende processen.

Niet alleen voor deze onderzoekslijn maar hier wel in het bijzonder, is een goede kennis van de **Life History Tactics** van de soorten van belang om experimenten op te zetten en in het interpreteren van de resultaten. Tenslotte moet benadrukt worden dat de keuze voor de te onderzoeken modelsoorten bepaald moet zijn door de onderzoeksvraag, en niet door de mate van zeldzaamheid of beschermingsstatus van de te onderzoeken modelsoort. Met andere woorden: een eventuele N2000 status van de modelsoort is geen leidend criterium in de keuze van de onderzoeksopzet.

10.2 Voedselkwaliteit en beheer

Om de effecten van toegenomen N-depositie uit het heden en de nabije toekomst op natuurkwaliteit zo veel mogelijk te mitigeren is het noodzakelijk om effectieve maatregelen te ontwikkelen. Dergelijke maatregelen zijn idealiter zo veel mogelijk gericht op het verwijderen van overtollig N uit het systeem en/of op het herstel van de natuurlijke buffercapaciteit, zonder dat dit van invloed is op andere systeemkenmerken en systeemkwaliteiten. Uit de beheerpraktijk blijkt dat dit op zijn minst een zeer grote uitdaging is. De meest effectieve maatregelen tegen N-ophoping zijn vaak ook erg ingrijpend en hebben daardoor vaak ook behoorlijke bijwerkingen op het functioneren en de soortenrijkdom van het systeem.

Voor het onderwerp van voedselkwaliteit is een **derde onderzoekslijn** noodzakelijk die uitgaat van het herstel van **nutriëntenbalansen**, **(micro)nutriëntgehalten en antivraatstoffen** in tegenstelling tot een herstel dat puur is gefocust op het creëren van N-arme omstandigheden. In deze onderzoekslijn moet de kennis opgedaan uit de **onderzoekslijnen naar veranderde processe**n worden gebruikt om maatregelen te ontwikkelen die de van nature geldende processen weer (gedeeltelijk) kunnen herstellen.

Een dergelijke aanpak zal in de praktijk vaak betekenen dat er meerdere ingrepen noodzakelijk zijn. Een voorbeeld is door het stimuleren van stikstofmineralisatie en nitrificatie de beschikbaarheid van anorganisch N tijdelijk te verhogen, waardoor tijdelijk de groei en N opname door planten gestimuleerd wordt. Door vervolgens (druk)begrazingsbeheer toe te passen kan de door de vegetatie opgenomen extra N worden afgevoerd, en door het sterk begrazen van de vegetatie wordt ook de uitspoeling van nitraat gestimuleerd. Uiteindelijk treedt hierdoor een reductie van de hoeveelheid anorganisch N in de bodem op (met name die van ammonium) waardoor plantensoorten die beter in staat zijn om anorganisch N te gebruiken worden bevoordeeld. Nitrificatie (en uitspoeling van nitraat) heeft echter als neveneffect dat de verzuringssnelheid toeneemt. Vervolgbeheer op dit aspect is dan weer nodig om tot het gewenste eindresultaat te komen. Dit voorbeeld gaat uit van effecten op bodemgesteldheid, maar het is evengoed mogelijk dat dergelijke onbedoelde beheereffecten ook direct op het niveau van producentkwaliteit optreden.

Vooral in **van nature laag-dynamische ecosystemen** en/of **systemen met van nature weinig menselijke invloed** is het van groot belang dat zij zo min mogelijk onbedoelde neveneffecten van beheeringrepen ondervinden. In de Nederlandse situatie vallen onder meer hoogveensystemen, vennen en bossen op (arme) zandgronden onder deze categorie. Een tweede groep zijn systemen die weliswaar sterk door menselijke invloed zijn gevormd of van nature enige dynamiek kenden (uitgeveende of anders door de mens beïnvloede vennen, stuifzanden, heiden, schraallanden, kalkarme duinen), maar die door tegenomen N depositie sterk in kwaliteit worden bedreigd en waar traditioneel beheer vaak niet meer afdoende is om de kwaliteit ervan te behouden. In deze ecosystemen is het van belang om na te gaan of bestaande (N-depositie mitigerende) maatregelen leiden tot onbedoelde verschuivingen in nutriëntenbalansen, en zo ja, hoe dit te voorkomen is of te mitigeren. Tevens ligt er voor deze systemen een uitdaging in het opstellen en toetsen van nieuwe beheermaatregelen. De in het recente verleden uitgevoerde en lopende onderzoeken naar

steenmeeltoepassing als alternatieve verzuringsbestrijdende maatregel is een goed voorbeeld van een mogelijk nieuw toepasbare maatregel gericht op het voorkomen van te sterke veranderingen in nutriëntbalansen. Het ontwerp en uitvoeren **van gerepliceerde beheerexperimenten in de veldsituatie**, eventueel aangevuld met **kasexperimenten** is hier de meest bruikbare onderzoeksmethode.

Deze **derde onderzoekslijn** richt zich dus met name op het effect van de beheermaatregelen op de **kwaliteit van producenten** en de **respons van consumenten** hierop. Aanvullende **metingen aan bodemprocessen** kan helpen in het verklaren van verschillen in producentkwaliteit, maar spelen hier uitdrukkelijk een dienende rol in het uiteenrafelen van de effecten hoger in de voedselketen.

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13 Appendices

Appendix 1. Number of relevant papers found per search string in the initial phase of literature research. These results provided the framework for further literature research, and is thus not representative for the total literature study.

Bijlage 1. Aantal relevante papers gevonden per zoekterm combinatie in de beginfase van het literatuuronderzoek. Deze resultaten vormden het kader voor verder literatuuronderzoek en zijn dus niet representatief voor de totale literatuurstudie.

Search string	Relevant papers (n)
thiamine+ecosystem+animal	6
riboflavine+ecosystem+animal	1
niacin+ecosystem+animal	0
tryptophan+ecosystem+animal	0
folic acid+ecosystem+animal	0
biotin+ecosystem+animal	0
panthotenic acid+ecosystem+animal	0
pyridoxine+ecosystem+animal	0
choline+ecosystem+animal	0
retinol+ecosystem+animal	1
vitamin E+deficiency+ecosystem+animal	0
vitamin D+ deficiency+ecosystem+animal	0
vitamin C+ deficiency+ecosystem+animal	0
cobalamine+ deficiency+ecosystem+animal	0
cobalt+ deficiency+ecosystem+animal	1
amino acid+ deficiency+ecosystem+animal	5
methionine+ deficiency+ecosystem+animal	0
proline+ deficiency+ecosystem+animal	0
non-protein amino acids+nitrogen+ecosystem+animal	6
linoleic/linolenic acid+deficiency+ecosystem+animal	6
sodium+deficiency+acidification+ecosystem+animal	1
chlorine+deficiency+acidification+ecosystem+animal	0
potassium+deficiency+acidification+ecosystem+animal	0
calcium+deficiency+acidification+ecosystem+animal	2
liming+acidification+ecosystem+animal	1
magnesium+deficiency+acidification+ecosystem+animal	0
manganese+deficiency+acidification+ecosystem+animal	0
phosphorus+deficiency+acidification+ecosystem+animal	0
phosphorus+deficiencies+nitrogen+deposition+ecosystem+animal	0
metal toxicities acidification ecosystem animal	0
zinc+deficiency+acification+ecosystem+animal	0
selenium+deficiency+acidification+ecosystem+animal	0

Appendix 2. *Species list of butterflies used in the butterfly trend analysis. Nutritional niche, host plant use and thermophily after Wallis de Vries (2014) and Wallis de Vries & Van Swaay (2017); post-1950 distribution trend shows relative change at 5x5 km resolution (Van Swaay, 2019).*

Bijlage 2. *Soortenlijst van vlinders gebruikt in de vlindertrendanalyse. Voedingsniche, waardplantgebruik en thermofiel naar Wallis de Vries (2014) en Wallis de Vries & Van Swaay (2017); De distributietrend na 1950 toont relatieve verandering bij een resolutie van 5 x 5 km (Van Swaay, 2019).*

Scientific name	Nutritional niche	Host plant use	Thermo-philous species?	Expected response	Post-1950 Distribution trend (%)
Agriades optilete	low	dwarfshrub	No	decline	-89
Boloria aquilonaris	low	dwarfshrub	No	decline	-80
Phengaris arion	low	dwarfshrub	Yes	decline	-100
Callophrys rubi	low	generalist	No	uncertain	-14
Plebejus argus	low	generalist	No	uncertain	-44
Plebejus idas	low	generalist	No	uncertain	-100
Coenonympha arcania	low	graminoid	Yes	decline	-100
Coenonympha tullia	low	graminoid	No	decline	-98
Hesperia comma	low	graminoid	Yes	decline	-78
Hipparchia statilinus	low	graminoid	Yes	decline	-94
Melitaea athalia	low	herbaceous	Yes	decline	-88
Phengaris alcon	low	herbaceous	No	decline	-89
Satyrium ilicis	low	woody	No	decline	-93
Carterocephalus palaemon	mesotrophic	graminoid	No	uncertain	+6
Coenonympha pamphilus	mesotrophic	graminoid	No	uncertain	-3
Heteropterus morpheus	mesotrophic	graminoid	No	uncertain	-42
Hipparchia semele	mesotrophic	graminoid	Yes	uncertain	-55
Ochlodes sylvanus	mesotrophic	graminoid	No	uncertain	+58
Thymelicus sylvestris	mesotrophic	graminoid	No	uncertain	-88
Boloria selene	mesotrophic	herbaceous	No	decline	-87
Erynnis tages	mesotrophic	herbaceous	Yes	decline	-95
Euphydryas aurinia	mesotrophic	herbaceous	No	decline	-100
Fabriciana niobe	mesotrophic	herbaceous	Yes	decline	-64
Lycaena tityrus	mesotrophic	herbaceous	Yes	decline	-72
Pyrgus malvae	mesotrophic	herbaceous	Yes	decline	-85
Speyeria aglaja	mesotrophic	herbaceous	No	decline	-88
Celastrina argiolus	indifferent	generalist	No	increase	+63
Aphantopus hyperantus	indifferent	graminoid	No	neutral to increase	+41
Maniola jurtina	indifferent	graminoid	No	neutral to increase	+29
Pararge aegeria	indifferent	graminoid	No	increase	+125
Pyronia tithonus	indifferent	graminoid	No	neutral to increase	+15
Thymelicus lineola	indifferent	graminoid	No	neutral to increase	+82
Lycaena phlaeas	indifferent	herbaceous	No	neutral to increase	+12
Gonpteryx rhamni	indifferent	woody	No	neutral to increase	+136

Appendix 3. *Number of sites included in the butterfly trend analyses for different combinations of soil buffering capacity and long-term annual average nitrogen deposition.*

Bijlage 3. *Aantal locaties die zijn opgenomen in de vlindertrendanalyses voor verschillende combinaties van bodembuffercapaciteit en langjarige jaarlijkse gemiddelde stikstofdepositie.*

Buffer capacity		Poor			Fair	
N deposition (mol·ha-1·y-1)	<2000	2000-2500	2500-3000	>3000	2000-2500	2500-3000
Species						
Callophrys rubi	6	38	44	8	63	24
Coenonympha pamphilus	11	46	87	23	100	91
Lycaena phlaeas	12	44	82	29	98	105
Lycaena tityrus	9	24	17	5	49	8
Maniola jurtina	17	67	122	42	153	148
Ochlodes sylvanus	16	57	115	36	135	132
Plebejus argus	8	38	52	8	60	20
Pyronia tithonus	12	29	80	27	78	90
Thymelicus lineola	14	52	88	39	124	119
Thymelicus sylvestris	6	24	42	16	71	51

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