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Reproductive Response of *Platynothrus peltifer* (C.L. Koch, 1839) to Continuous Nitrogen Deposition

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Abstract: Continuous nitrogen deposition threatens ecosystems by acidifying soils, causing a stoichiometric imbalance in the vegetation and ultimately, the disappearance of plant and animal species. There is a gap in knowledge of how decomposers such as oribatid mites cope with the effects of nitrogen deposition. Therefore, we conducted feeding experiments with the herbivorous mite *Platynothrus peltifer* (C.L. Koch, 1839) to assess its fitness as a measure of its reproductive response towards different nitrogen levels in its diet. Mites were collected from the field, starved, and allowed to lay eggs. We recorded the number of eggs during 60 days of experimental trial. The fecundity of mites varied with different elemental compositions, whereby phosphorus seemed to be a limiting factor. With ongoing nitrogen deposition in the future and concomitant phosphorus limitation, we expect a negative impact on the population dynamics of herbivorous decomposers such as *Platynothrus peltifer*.

Keywords: nitrogen deposition; stoichiometry; acidification; decomposition; Oribatida; phosphorus limitation

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1. Introduction

Nitrogen deposited in the environment by human activities increases the availability of nitrogen in a naturally nitrogen-limited system [1,2]. This leads to two main issues for the environment: first, it accelerates the plant growth of the overall vegetation. The rapidly growing plants also absorb all the remaining nutrients in the soil, storing them in organic biomass. At the same time, the soil pH drops rapidly due to the additional nitrogen, and the environment becomes more acidic. Decomposition is thereby slowed down, and in a soil environment of pH 4.2 or lower, aluminium and iron that have been stably bound to minerals come free [3]. Aluminium in a free ionised form harms plant roots and binds together with iron phosphorus from the soil to form insoluble phosphates, such as $AlPO_4$ and $FePO_4$ [4,5]. Continuous nitrogen deposition harms the environment over the long term and increases phosphorus limitation in soil ecosystems [6]. Consequently, many animal species from various taxa and plant species disappeared from the altered habitat [7–9].

The consequences of nitrogen deposition have come to effect in the Veluwe and pushed the ecosystem into a new state of blocked nutrient cycling. The oldest oak forests on the Veluwe, in the Netherlands in nutrient-poor sandy soil, are now suffering from a more pronounced phosphorus limitation than before in an already phosphorus limited system [10]. The vegetation became imbalanced in its elemental stoichiometry, meaning that all plant material increased in its nitrogen-to-phosphorus ratio. As a result, animals struggle to take up sufficient amounts of phosphorus, while due to their compensatory feeding strategy, they stop feeding when they have enough nitrogen [11]. Previous studies

with insect herbivores showed that imbalanced stoichiometry decreases overall fitness, growth, and overall reproduction rate [9]. Correspondingly, the organic matter layer stacks up since decomposing organisms are not suited for the altered, acidic soil environment [12].

However, not all decomposers may be equally affected by these harsh conditions, as micro-arthropods are very resilient soil organisms. They are present in nearly every soil ecosystem with a Holarctic distribution. Micro-arthropods reach densities of up to 166×10^4 individuals per m^2 in temperate forests and live predominantly in the litter and the first 5 cm of the topsoil [13]. To understand their ecological role in the soil system, micro-arthropods have been categorized into feeding guilds based on their enzymatic activities [14]. Herbivorous micro-arthropods contribute directly to decomposition through organic matter fragmentation and feeding, while fungivores feed on the decomposing fungi and enhance their growth [15,16]. Micro-arthropods that feed on plant material directly, are especially directly exposed to this stoichiometric imbalance in the vegetation and could possibly suffer fitness disadvantages.

To answer this question, this paper studies the oribatid mite *Platynothrus peltifer*, an asexual herbivorous grazer. We chose this species based on its feeding guild, its high abundance in nearly every soil, and its intermediate size of 865 µm, since bigger mite species appeared to be absent in phosphorus-limited forests. Its ability to starve for about 2 months without increased mortality makes it a suitable candidate for these experiments [14,17,18].

Numerous studies have demonstrated that *Platynothrus peltifer* responds to heavy metal pollution in its diet by reducing egg production [19]. However, there is a lack of information regarding the specific nutrients crucial to optimal egg production in this species. This gap in knowledge leads us to our central research question, as follows: How do nitrogen deposition and subsequent increases in nitrogen levels in the vegetation affect the egg production of *Platynotrhus peltifer*? We hypothesise that *Platynothrus peltifer* will produce fewer eggs when exposed to diets with high nitrogen and low phosphorus levels. This is based on findings from previous insect feeding studies, which have identified phosphorus as a critical nutrient [20].

2. Materials and Methods

For this experiment, the oribatid mite *Platynothrus peltifer* (C.L. Koch, 1839) was collected from the field and kept in Petri dishes for a feeding trial of six different food types, plus a negative control without food. Food consisted of dried leaves from an oak and an aspen tree, each with three different seasons of leaves, varying in nutritional contents such as nitrogen, carbon, and several trace elements. During 60 days of feeding experiments, the number of laid eggs was recorded as a measure to connect nutrition with reproduction success.

2.1. Collection of Specimens

Platynothrus peltifer is an herbivorous grazer that can best be found in litter layers of deciduous trees and the vegetation layer of moss of the class Bryophyta. The collection of mosses of the species *Hypnum jutlandicum* and *Thuidium tamariscinum* proved to be most successful during the experiments [17]. Samples were taken in the Heumersoord Forest in Nijmegen (51°48′25.3″ N, 5°52′18.2″ E). The Heumersnoord Forest is a coniferous dry forest that is maintained for wood production. It includes different biotopes, such as heathland, dunes, and mixed-forest parts of coniferous and deciduous tree species. All samples were taken randomly under tree-covered, shaded, and moist areas around the given coordinates. Mosses of the species *Hypnum jutlandicum* and *Thuidium tamariscinum* were collected to extract mites.

Following a Berlese–Tullgren approach to extraction [21,22], moss was placed upside down on a round manual sieve with different mesh sizes between 0.8 and 2 mm and a sieve pan filled with tap water. Sieves were left to dry in a light room under a potassium light bulb for at least 48 h. This slow drying process ensured that the mites had enough time to escape the heat and migrate towards the water bowl, where they could be collected. Live

Platynothrus peltifer could easily be recognised by its dark brown colour, two dorsal ridges, size, and the fact that it sank to the bottom in the sieve pan.

All mites were collected in one 55 mm ∅ plastic Petri dish with glass fibre filter paper and tap water until experiments began. They were stored in a climate chamber at 11 °C and 75% humidity. Mites did not receive any food until the start of the experiments, since *P. peltifer* can endure long periods without food [17]. All mites were, on average, starved for 14 days before the start of the feeding trial.

2.2. Experimental Conditions in the Climate Chamber

All experiments were carried out in 55 mm ∅ plastic Petri dishes without notches, containing a 50 mm-diameter glass fibre filter paper (Brand: Wattman, not bleached) to ensure moisture. The Petri dishes were placed with closed lids (not airtight) in a 17 °C climate chamber at 75% humidity and a 14 h light/10 h dark rhythm to mimic summer conditions during the whole 60 days of the experiment. The Petri dishes were examined under a stereomicroscope with a magnification of 10–40× to investigate for laid eggs. This experimental setting is based on experiments from Siepel [17] and was modified to ensure standardisation.

2.3. The Food Treatments

To mimic real food conditions, leaves from two trees, *Populus tremula* L. (1753) (P) and *Quercus robur* L. (1753) (Q), were used. Both tree species are typically found in Dutch forests; the summer oak *Quercus robur* L. (1753) has been kept in the Veluwe for wood economy, while the *Populus tremula* L. (1753) tree can be found in smaller forest stands, such as the Gelderse port or close to the German border. The leaves were collected in 2021 directly from the trees during different seasons, namely, spring, summer, and autumn in the Veluwe area near Beekbergen (52°08′57.0″ N 5°57′29.8″ E for the oak leaves and 52°10′03.1″ N 6°00′41.1″ E for the poplar leaves). Leaves were collected at heights of 1.5–2 m directly from the tree; the trees did not show any signs of insect herbivory or pests. The leaves were oven-dried for 24 h at 70 °C, ground to powder, and stored in airtight plastic vessels until use. In total, that makes the following 6 food treatments: PV, PZ, and PN from *Populus tremula* for spring, summer, and autumn, as well as the other three treatments, QV, QZ, and QN, the same with leaves from *Quercus robur.* Elemental values of the food treatments can be found in Section 3.

To check for eggs laid based on stored energy resources, one treatment control was kept moist at the same condition as the other Petri dishes but did not receive any food.

2.4. Experimental Handling

The experiments lasted 60 days in total, during which mites were fed twice, once on day 0 at the beginning of the trial and the second time on day 30 with excess food (measured in a mite spoon), while faeces were not removed from the Petri dishes.

The Petri dishes were checked approximately once a week for new eggs. Found eggs were recorded with the day of the experiment and placed into a new Petri dish, and dead mites were recorded and discarded. A mite was considered dead when it did not move anymore and either (a) had its legs fall off when touched with a needle, (b) had its internals everted outside the anal plate, or (c) did not move after 2 min of touching it. Petri dishes were checked under a stereo microscope with a magnification of 10–40 times. Eggs were relocated with a small painting brush.

In total, 350 mites were used in the feeding trial, distributed over 7 treatments (6 leaf treatments + control), each with 5 replicates per treatment and 10 mites per Petri dish.

2.5. Nutritional Analysis

Nutritional analysis was carried out for the food treatments (PV, PZ, PN, QV, QZ, QN) right before the food trials were started. Carbon and nitrogen content were measured on a vario microcube analyser in total percentages of the sample and transformed into

PPM. Trace elements, such as calcium, iron, potassium, magnesium, manganese, sodium, sulphur, silicon, phosphorus, and zinc, were measured on ICP-OES (iCap 6300) in PPM. Phenols were measured in the facilities of the University of Amsterdam, following the Folin Ciocalteu phenol protocol (see Supplementary Materials) [23].

2.6. Statistical Data Handling

Data were processed in the program R Studio, and parts of the script were adapted from Joost Vogel's data analysis [20,24].

Further, the data analysis of this paper is mainly focused on linking the nutritional data of the food treatments with the help of models with the number of eggs laid.

To also account for deaths for later data processing, the unit mite days were calculated based on the egg-laying data over time. It contains the number of live mites per each time interval of checking for eggs, separately, and has been summed up to the unit mite days sum, which accounts for all mites that were alive and present during the experiment per Petri dish. For example, if 10 mites were present during the whole 60 days, this Petri dish has a 600 mite days sum.

Based on the mite days sum, the productivity was calculated as follows: the number of total eggs divided by the mite days sum; therefore, all the eggs were put into perspective for live mites.

The unit productivity is used for the linear models to link nutrients to a positive or negative contribution to egg production since it saves one degree of freedom of mortality that is already included in the unit productivity. Based on AIC values and VIC for explanatory variables, linear models with the best fit to explain productivity have been chosen (see Tables A5 and A6 for complete model lists and *p*-values).

In the following sections, food treatments in this experiment are addressed with their abbreviations as introduced in Section 2.3. For a clearer overview, a table is provided below with the abbreviations and the types of food treatments they contain.

3. Results

3.1. Food Quality

Each food treatment has been measured for elemental composition, and values have been transformed into PPM. Nutrients and trace elements have been further checked for correlation and identified for influence on productivity by means of linear models (see Appendix A). In the case of highly correlated elements, we took care not to include both elements in a single model (see Appendix B).

In general, we observed a clear seasonal pattern in nutritional contents and trace element contents across our leaf treatments, with either an increasing or decreasing trend through the seasons from spring to summer to autumn.

Leaves from the spring season show poplar tree treatments with the highest amount of nitrogen, with summer being the next lowest and autumn having the lowest value for the treatments (Figure 1A). For the oak leaves, this pattern is disrupted by leaves from summer having the highest nitrogen, then spring and autumn in decreasing order.

Phosphorus content decreased in all treatments over the season (Figure 1B), with poplar tree treatments having higher phosphorus values than the oak treatments, while oak leaves from summer also showed the lowest phosphorus content among all treatments.

For phenolic content, the summer treatment of both trees showed the lowest values, followed by spring, with slightly higher values, and autumn, with the highest phenolic content, while leaves from the oak tree have an overall higher phenolic content than those of the poplar tree (Figure 1C).

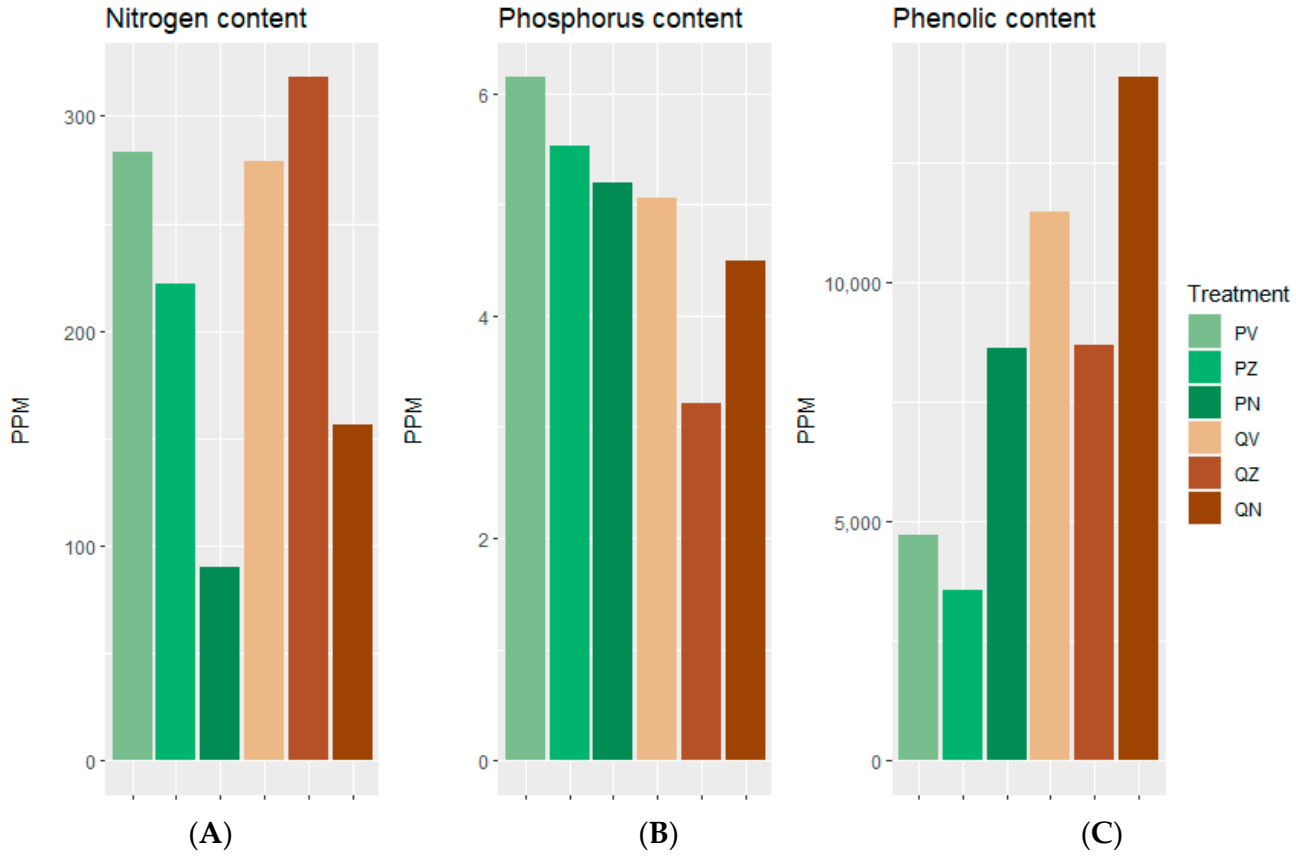

Figure 1. Nitrogen (**A**), phosphorus (**B**), and phenolic content (**C**) measured in PPM for all food treatments; see Table 1 for abbreviation explanation.

Table 1. Explanation of used abbreviations for food treatments.

PV	Populus tremula leaves—spring
PZ	Populus tremula leaves—summer
PN	Populus tremula leaves—autumn
QV	Quercus robur leaves—spring
QZ	Quercus robur leaves—summer
QN	Quercus robur leaves—autumn
Con	Control treatment (has not received any food during the experiments)

For potassium, all leaf treatments decreased from spring, which had the highest amounts of potassium, to autumn, which had the lowest amounts of potassium, with the poplar tree having higher potassium values than the oak tree (Figure 2A). For silicon, the pattern is the opposite; spring was shown to have the lowest amount, increasing equally per treatment towards the autumn treatments, which had the highest amount of silicon, while the poplar tree has higher values of silicon compared to the same seasonal treatment (Figure 2B).

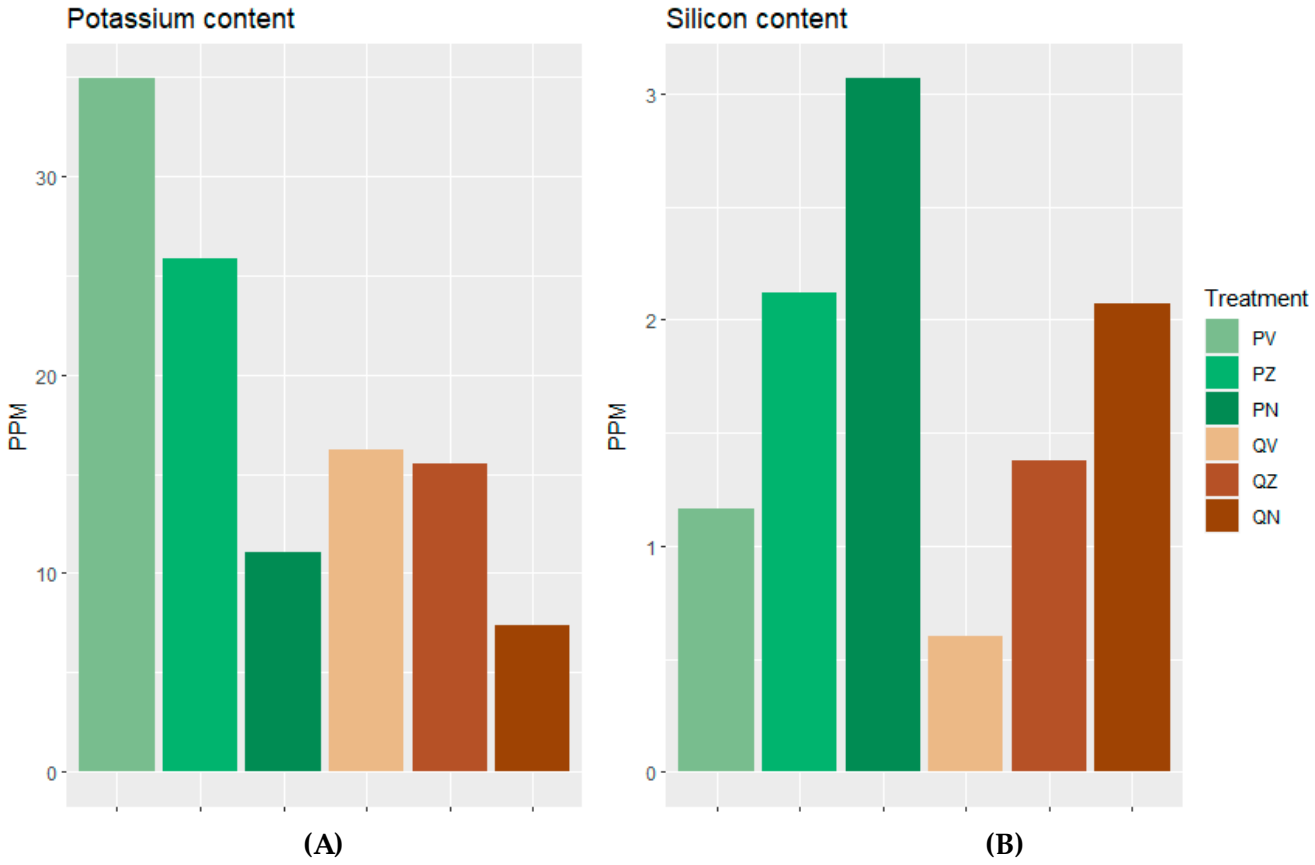

Figure 2. Potassium content (**A**) and silicon content (**B**) measured in PPM for all food treatments; see Table 1 for abbreviation explanation.

3.2. Mite Fecundity

Across treatments, mites that fed on spring leaves from poplar (treatment PV) produced more eggs compared to the control treatment; treatment PV was significantly more productive in comparison to the control treatment, PZ, PN, and QN (Figure 3, pairwise *t*-test with Bonferroni correction, p.adj < 0.02). In fact, treatment PV was the most productive treatment, with an average number of 74.2 eggs ± 18.01 sd (control 39.4 ± 5.03 sd). For both trees, *Populus tremula* and *Quercus robur*, the most productive treatments were spring leaves, followed by summer and autumn.

To explain the reproduction rate from leaf nutrients and elemental content, the best linear model among different candidates appears to be carbon (C), nitrogen (N), and phosphorus (P), whereby phosphorus and nitrogen separately contribute positively to the productivity, and carbon contributes negatively (see Tables A5 and A6). When used as a combined ratio, for example, CN, NP, or CNP, the ratio appears in the model to contribute positively to productivity.

Phenolic content appears to always contribute negatively to different combinations with different nutrients (see Tables A5 and A6).

For the elements, only potassium (K) and silicon (Si) were shown to have a significant effect on the productivity in single linear models (lm productivity~potassium + silicon, p value < 0.05, R-adj = 0.4471); other element candidates have been excluded based on a correlation matrix (see Table A7).

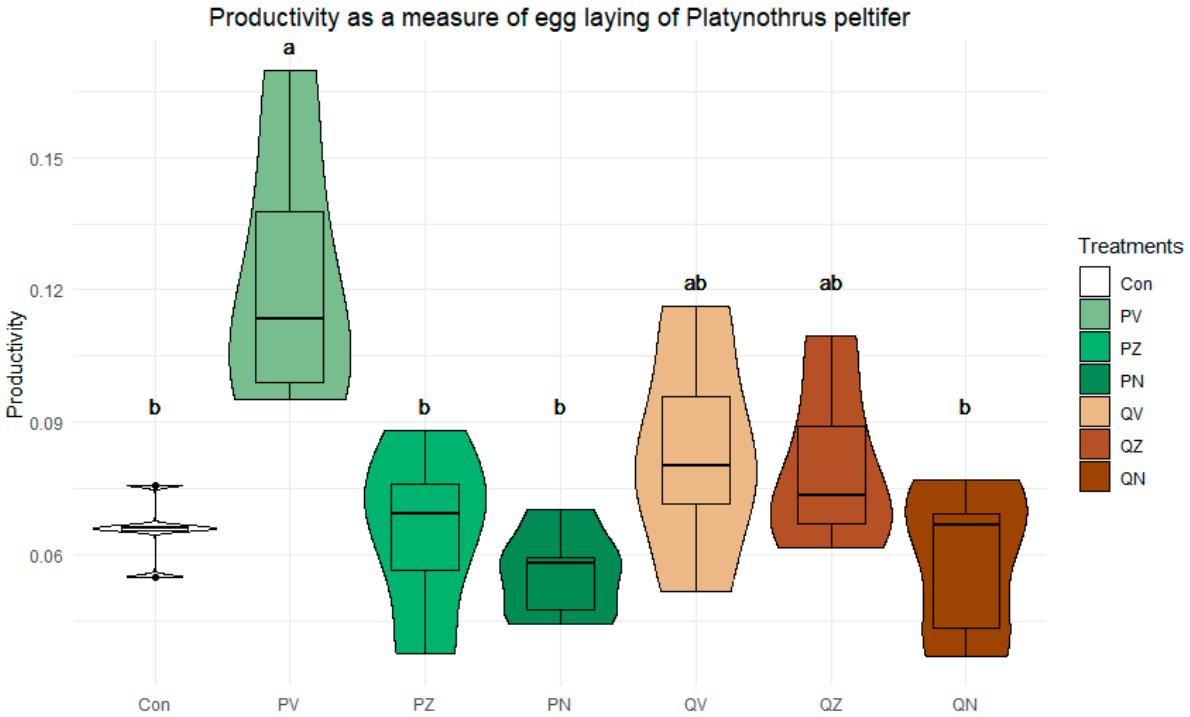

Figure 3. Violin boxplot per treatment, showing the productivity as a measure of laid eggs, including time and mortality as components; letters indicate significant differences of treatments to one another; see Table 1 for abbreviation explanation.

4. Discussion

The results show that productivity as a measure of laid eggs by *Platynothrus peltifer* is influenced by interactions between carbon, nitrogen, phosphorus, and phenolic content in different ways.

In all models, carbon showed a negative contribution to productivity. The total content of carbon in foliar material is provided in various molecular ways, mostly as structural molecules such as cellulose and lignin [25]. *Platynothrus peltifer* was shown to have both enzymes to digest cellulose and lignin [14]. Nevertheless, the consumption of those structural molecules is intensive in energetic costs due to these specific enzymes for digestion and less preferred by herbivores [26]. All *Quercus robur* treatments were shown to have the highest amounts of carbon and phenolics, as well, compared to leaves from *Populus tremula* within the same season. The detrimental combination of possibly hard-to-digest carbon sources and phenols may have led to lower production of eggs due to inefficient energy uptake.

In all models, nitrogen appears to have had a positive contribution towards productivity as a measure of laid eggs. Nitrogen plays a pivotal role in various metabolic processes within animals by being integrated into essential amino acids, which are the building blocks of proteins and enzymes. This incorporation is essential for the synthesis and function of these vital biological molecules [27]. Regardless of showing higher nitrogen contents than average for European trees (see Appendix D, [12,28,29]), it still shows to have a positive effect. Following the studies by Mellert and Göttlein [30], leaves from *Quercus robur* in this experiment were shown to have surplus nitrogen content—up to extreme levels of nitrogen (see Appendix D for harmonised values into mg/g); however, possessing phosphorus levels according to the present concentrations still appears to have a positive contribution to egg production. Additionally, nitrogen levels may have surpassed critical thresholds for herbivores, as evidenced by treatment QZ, which exhibited the highest nitrogen content but did not yield the highest productivity among all food treatments.

As a result, this leads to the conclusion that phosphorus could be the limiting element for egg production since food treatment PV had the overall highest productivity and the highest phosphorus content (see Figure 1). Several studies by Vogels et al. [8,9,20] show that invertebrate herbivores performed better relating to metabolic processes like growth or egg production with food enriched in P in comparison with low P content and can be found in higher abundances and diversities in P-richer soil. Very little is known about the metabolic requirements of herbivorous Acari since they belong to the taxa of arachnids, which appear to be mostly predatory or parasitic.

From all measured trace elements, potassium and silicon were shown to have a significant effect on productivity; potassium was shown to have a positive influence and silicon was shown to have a negative influence. Generally, it is known that potassium is needed on a cellular level for muscular locomotion [27], but further, it is also known that spiders need potassium for web production, and it appears in high concentrations in their venom [31,32].

Silicon, on the other hand, is known to have a protective effect against herbivory in grasses, in which it damages the mouthparts of arthropod herbivores [33]. This finding aligns with the negative impact of silicon on productivity; the autumn leaves were shown to have the highest silicon concentration and were, in comparison with leaves from the same tree, shown to have the lowest productivity.

Generally speaking, mites seem to have eaten the offered food, since faeces aggregated in the experimental Petri dishes and food piles visually disappeared over time. In the control treatment without any food, white, crystal-like faeces appeared, which leads to the assumption that they tried to eat the transparent glass fibre filter paper. The fact that mortality was not significantly higher in the control treatment than in the other food treatment, glass fibre passing their digestion does not seem to affect them. Mites seem to be unable to discriminate between food items they encounter. In the underground life of the soil, in which all kinds of food items appear for the rather unselective feeder herbivorous grazer mite, this strategy can work perfectly well and does not make discriminating between food items necessary, as the digestive capacity here determines the nutrient uptake [14,34,35].

5. Conclusions

We showed that egg production in *Platynothrus peltifer* responded to the different food treatments. Variations in egg production across food treatments could be related to differences in carbon, nitrogen, phosphorus, and phenolic contents in the leaves provided. Although these leaf characteristics covary, our models indicated phosphorus as a limiting element. Other elements, such as potassium and silicon, could also help explain the variation in egg production, but to a lesser extent. However, to pinpoint more precisely which elements are essential for egg production, more experiments using artificial food treatments are needed. Mites fed with leaves from *Populus tremula* from the spring were shown to have the highest egg production. These leaves had the highest phosphorus content, second-highest nitrogen content, and second-lowest phenolic content. With high values of potassium and low values of silicon, they appear to be the optimal mix in this feeding experiment.

With the ongoing nitrogen deposition, higher levels of nitrogen in the environment and in plant material can be expected for the coming future [36]. The acidification of the already nutrient-poor sandy soils will increase, and elements such as phosphorus will be less available chemically in the soil, which will eventually lead to stoichiometrically imbalanced vegetation. We already know that many different invertebrate herbivores suffer fitness loss due to imbalanced food. [7,20,37], and therefore, we can expect to see a change in the egg production of *Platynothrus peltifer* and possibly other herbivores in the increasingly P-limited forests.

Supplementary Materials: The supporting material is accessible under https://zenodo.org/records/11488404?token=eyJhbGciOiJIUzUxMiJ9.eyJpZCI6IjI0YjYxYzVjLTM2OGEtNGFkYS05NzRmLTY3ZjU0ZGE5ODhlOSIsImRhdGEiOnt9LCJyYW5kb20iOiJlN2Q0Yjg1YzIwZTQyNWYwMDM0ZDM1ODYyMjgxYWFjMyJ9.BKOI7SN5yWOIkL4To8TZuzDL8Fh0DIgI32uJu9tx0_xSj9m6l3D1I7aFTCPHspXF8D1FbwXQmxKTFuEl66lxeA (accessed on 1 June 2024). In Mitedata.xlsx table 1: PlathynothrusPeltifer, table 2: SumEggsPPeltifer; in Nutrients.xlsx table 1:Sheet1, table 2: N P %, table 3: Calculation, table 4: Explanation; in LmSummary table 1: PreAnalysisNutrients, table 2: CorMatrix, table 3: CorMatrix-CNP, table 4: LmRequirements, table 5: CNP Models, table 6: Trace Elements, table 7: Deaths.

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Data Availability Statement: The data that support the findings will be archived open access in the data repository Zenodo upon acceptance for publication. For the review process, we have included the dataset in a temporary Appendix as a supporting file for the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Correlation matrix for carbon (C), nitrogen (N), phosphorus (P), and phenols (Phenols_mgL) from food treatments, calculated in R with the cor() command, outputting Pearsons's correlation coefficient between the measured elements.

	C	N	P	Phenols_mgL
C	1	0.536502	−0.61159	0.654046
N	0.536502	1	−0.20767	−0.25218
P	−0.61159	−0.20767	1	−0.49191
Phenols_mgL	0.654046	−0.25218	−0.49191	1

Appendix B

Table A2. Correlation matrix calculated with the cor() command in R, outputting Pearson's correlation coefficient between measured elements, using raw PPM data (number after the element is the wavelength the element has been measured in).

	Ca3179	Fe2599	K_7664	Mg	Mn2576	Na5889	S_1820	Si2516	Zn2138
Ca3179	1	0.794381	0.028697	0.955909	−0.06406	0.761366	0.601126	0.835905	0.934073
Fe2599	0.794381	1	−0.40242	0.614766	0.385577	0.796924	0.231138	0.947602	0.780686
K_7664	0.028697	−0.40242	1	0.314619	−0.78129	−0.44061	0.725328	−0.37597	−0.05156
Mg	0.955909	0.614766	0.314619	1	−0.297	0.610013	0.760679	0.6728	0.880869
Mn2576	−0.06406	0.385577	−0.78129	−0.297	1	0.327982	−0.60925	0.472874	−0.00593
Na5889	0.761366	0.796924	−0.44061	0.610013	0.327982	1	−0.02678	0.853558	0.904203
S_1820	0.601126	0.231138	0.725328	0.760679	−0.60925	−0.02678	1	0.210128	0.391889
Si2516	0.835905	0.947602	−0.37597	0.6728	0.472874	0.853558	0.210128	1	0.830888
Zn2138	0.934073	0.780686	−0.05156	0.880869	−0.00593	0.904203	0.391889	0.830888	1

Appendix C

Table A3. Levene test result to check the homogeneity of productivity as a response variable.

Formula: Levene.Test (Productivity~Treatment)			
statistic	*p*-value	df	df.residual
1.052194	0.413868	6	28

Table A4. Test results of the Shapiro–Wilk normality test on the residuals on the linear model with productivity~treatment.

(Intercept)	C	Model_Name	N	P	NP	CN	CNP	C:N:P	N:P	C:N	Phenols
0.064865	2.86×10^{-6}	lmCNP1	NA	NA	NA	NA	NA	NA	NA	NA	NA
0.052925	NA	lmCNP2	0.00012	NA	NA	NA	NA	NA	NA	NA	NA
0.06017	NA	lmCNP3	NA	0.003749	NA	NA	NA	NA	NA	NA	NA
0.066675	-8.06×10^{-6}	lmCNP4	0.000212	NA	NA	NA	NA	NA	NA	NA	NA
0.063544	-3.88×10^{-6}	lmCNP5	NA	0.006537	NA	NA	NA	NA	NA	NA	NA
0.053706	NA	lmCNP6	0.000124	−0.00036	NA	NA	NA	NA	NA	NA	NA
0.064686	NA	lmCNP7	NA	NA	0.000272	NA	NA	NA	NA	NA	NA
0.088012	NA	lmCNP8	NA	NA	NA	−0.00058	NA	NA	NA	NA	NA
0.090213	NA	lmCNP9	NA	NA	NA	NA	−0.0033	NA	NA	NA	NA
0.065932	-5.16×10^{-7}	lmCNP10	NA	NA	0.000291	NA	NA	NA	NA	NA	NA
0.064352	NA	lmCNP11	NA	0.009386	NA	−0.00135	NA	NA	NA	NA	NA
0.04864	NA	lmCNP12	NA	NA	NA	NA	NA	6.38×10^{-9}	NA	NA	NA
0.048713	NA	lmCNP13	NA	NA	NA	NA	NA	NA	2.91×10^{-5}	NA	NA
0.053881	NA	lmCNP14	NA	NA	NA	NA	NA	NA	NA	2.50×10^{-8}	NA
0.064893	-2.00×10^{-5}	lmCNP15	0.000242	0.010069	NA	NA	NA	NA	NA	NA	NA
0.065837	NA	lmCNP16	NA	0.005633	NA	NA	NA	NA	NA	NA	-1.86×10^{-6}
0.062315	NA	lmCNP17	0.000151	NA	NA	NA	NA	NA	NA	NA	-2.10×10^{-6}
0.059825	NA	lmCNP18	0.000138	0.001453	NA	NA	NA	NA	NA	NA	-2.26×10^{-6}
0.0632	9.20×10^{-6}	lmCNP19	NA	NA	NA	NA	NA	NA	NA	NA	-3.16×10^{-6}
0.087187	NA	lmCNP20	NA	NA	NA	−0.00062	NA	NA	NA	NA	2.42×10^{-7}
0.073158	NA	lmCNP21	NA	NA	0.000378	NA	NA	NA	NA	NA	-1.76×10^{-6}
0.087785	NA	lmCNP22	NA	NA	NA	NA	−0.00438	NA	NA	NA	9.64×10^{-7}

Table A5. Overview table of linear models to explain productivity as a measurement of laid eggs with different explanatory variable combinations. The code to produce this table can be found in R code in Supplementary Materials section of this paper. Intercept column gives the value for intercept in the model summary, model_name for the name used in the R code; numeric values in all other columns are *p*-values from the model summary for the corresponding element. When the element has not been part of the model, an NA is given in the table. Following R syntax: for interaction between elements, NP, CN, and CNP are calculated elemental ratios between those elements.

Productivity		
statistic	*p*-value	method
0.901372	0.004305	Shapiro-Wilk normality test

Table A6. Overview table of linear models, with productivity as a measure of eggs as the responsive variable with additional information about model fit, ordered for increasing values of delta AIC; see Supplementary Materials R code for more information on how the table was created. df = degrees of freedom from model summary, AIC = Akaike information criteria, deltaAIC = difference between calculated AIC, rel.L.L = relative likelihood, see R code, weights = AIC weights; VIF = separately calculated variance inflation factor from car package (only calculated for linear models with explanatory variables ≥ 2, whereby both variables receive the same VIF, while every variable receives its own VIF within the model when 3 or more explanatory variables are included, table borders mark affiliation of VIF to the model name. See Table A5 for model components. order of VIF = order of explanatory variables in the linear model call.

	df	AIC	deltaAIC	rel.LL	Weights	VIF
lmCNP15	5	−160.876	0	1	0.356872	**6.356318083**
lmCNP12	3	−159.651	1.224735	0.542066	0.193448	**4.028078**
lmCNP13	3	−159.505	1.37071	0.503911	0.179832	**2.541963**
lmCNP11	4	−158.922	1.95342	0.376548	0.134379	1.525181185
lmCNP17	4	−156.863	4.012831	0.13447	0.047989	1.142255696
lmCNP4	4	−156.196	4.679736	0.09634	0.034381	2.432755632
lmCNP18	5	−155.172	5.703169	0.057753	0.02061	**1.249905996**
lmCNP2	3	−153.964	6.911978	0.031556	0.011261	**1.570094**
lmCNP14	3	−153.607	7.268588	0.026403	0.009422	**1.68696**
lmCNP6	4	−151.981	8.894397	0.011711	0.004179	1.54166742
lmCNP19	4	−149.441	11.43453	0.003289	0.001174	2.033455996
lmCNP9	3	−149.346	11.52989	0.003136	0.001119	
lmCNP21	4	−149.13	11.7458	0.002815	0.001004	1.173931567
lmCNP16	4	−148.812	12.06374	0.002401	0.000857	1.227276607
lmCNP8	3	−148.378	12.49732	0.001933	0.00069	
lmCNP7	3	−148.359	12.51628	0.001915	0.000683	
lmCNP3	3	−147.882	12.99387	0.001508	0.000538	
lmCNP22	4	−147.872	13.00381	0.001501	0.000536	1.849768094
lmCNP20	4	−146.418	14.45747	0.000725	0.000259	1.482416442
lmCNP1	3	−146.408	14.46712	0.000722	0.000258	
lmCNP10	4	−146.379	14.4965	0.000711	0.000254	1.71459025
lmCNP5	4	−146.375	14.50064	0.00071	0.000253	3.855024085

Table A7. Linear model result to explain productivity as a measure of laid eggs, with potassium (K) and silicon (Si) as explanatory variables; see R script for full list of tested linear models to check for a significant explanation of productivity data.

lmNut2, Formula: lm(Productivity ~ Potassium + Silicon)					
term	estimate	std.error	statistic	*p*-value	Adj. R Square/VIF
(Intercept)	0.067262	0.00793	8.482399	1.08×10^{-9}	0.4471
K_7664	0.001634	0.000334	4.900563	2.65×10^{-5}	1.023448
Si2516	−0.0115	0.003765	−3.05468	0.004516	1.023448

Appendix D

Table A8. Overview table with nitrogen (N) and phosphorus (P) contents in different units to compare with the literature values.

Sample	P PPM	P in %	P in mg/g Sample	N %	N mg/g Sample	N in PPM	N/P Ratio
QV	6.145	0.06145	0.6145	2.79	27.9	279	45.40277
QZ	5.522	0.05522	0.5522	3.18	31.8	318	57.58783
QN	5.202	0.05202	0.5202	1.56	15.6	156	29.98847
PV	5.056	0.05056	0.5056	2.83	28.3	283	55.9731
PZ	3.216	0.03216	0.3216	2.22	22.2	222	69.02985
PN	4.498	0.04498	0.4498	0.9	9	90	20.00889

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