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Earthworms increase soil mineral nitrogen content – a meta-analysis

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Abstract

Soil organisms and their interactions play a key role in various ecosystem processes and functions, such as the provision of nutrients. The main actors in nitrogen transformation processes are microorganisms, but earthworms affect these processes as their activity results in changes of the microhabitat and microbial community. Studies have shown that nitrogen content is higher in earthworm casts than in bulk soil, and that earthworm invasion affects soil mineral nitrogen. However, we still lack a quantitative synthesis of earthworm effects on soil nitrogen in bulk soil that integrates the influence of potential controlling factors (i.e., soil properties, climatic conditions and experimental parameters). Here, we investigated the impact of earthworms on soil ammonium (NH_4^+), nitrate (NO_3^-) and total mineral nitrogen (ammonium + nitrate, N_{min}) using meta-analytic techniques. Earthworms generally increased NO_3^- (+ 88 %) and N_{min} (+ 63 %), but did not affect NH_4^+. We assume that earthworms affect total mineral nitrogen mainly by their impact on NO_3^-. Endogeic and epigeic earthworms significantly increased NO_3^- and N_{min}, whereas no clear effect of anecic earthworms was found. This result is presumably caused by diverse effects of the different ecological groups on the microbial community composition. Our results for mixed ecological groups (i.e., anecic + endogeic earthworms) imply that anecic earthworms can lessen the effect of endogeic earthworms. The impact of earthworm presence on NO_3^- and N_{min} increased when experiments lasted longer than one week. The effect of earthworms on NH_4^+, NO_3^- or N_{min} was not influenced by earthworm abundance and biomass, soil organic carbon, soil C:N ratio, litter C:N ratio, the initial amount of NH_4^+, NO_3^- or N_{min}, total soil nitrogen or temperature. However, as data availability or replication across factor categories was low for some of these moderators, the non-significant results should be interpreted with caution. Also, we could not investigate interactions among the controlling factors due to paucity of data. Our study thus reveals important knowledge gaps regarding earthworm effects on soil nitrogen. For instance, only few or no data are available for effects of anecic earthworms, or earthworm effects in clay or sandy soils. Also, information on soil properties (e.g. soil organic carbon, soil or litter C:N ratios) are often not reported. Finally, observations of more complex earthworm communities are missing. Overall, our results highlight the importance of earthworms for soil nitrogen cycling and strengthen the call for soil-functional models to incorporate soil faunal effects.

Keywords Ecological groups | inorganic nitrogen | mineralization | nitrogen cycle | soil functions

1. Introduction

Soils provide numerous functions that are essential to ecosystem services, such as the provision of nutrients, which is the basis for plant growth and food and fiber production (Brussaard 2012). Among the soil fauna, especially earthworms are acknowledged for their role in ecosystem functions and services as they shape the soil structure, protect organic material against mineralization as it is mixed with soil particles, promote water infiltration, litter decomposition and nitrogen mineralization (Blouin et al. 2013) and dominate the

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biomass of soil fauna in most biomes (Fierer et al. 2009). Despite the importance of soil fauna for soil fertility (Osler 2007, Groenigen et al. 2014), nitrogen levels in agricultural soils are mainly adjusted by mineral fertilizer application. However, only around half of the globally applied nitrogen is taken up by plants, whereby the remainder is lost from the soil system by leaching, erosion or gaseous emission (Liu et al. 2010). This has profound environmental and economic consequences, and a more sustainable nitrogen management is needed. Modeling approaches and science-based decision support tools may help to improve fertilizer recommendations (Setiyono et al. 2011, Helming et al. 2018, Vogel et al. 2018, Rurinda et al. 2020). Although the key actors for nitrogen transformation processes are microorganisms, 30 % of nitrogen mineralization is assigned to soil faunal activity (Neher & Barbercheck 1998). In contrast, most models of nitrogen cycling do not incorporate the role of soil fauna (Osler & Sommerkorn 2007, Salo et al. 2016). The improvement of models by the integration of soil fauna requires a comprehensive understanding of their role in nitrogen cycling and a quantification of soil faunal effects. To this end, we assessed the impact of earthworms on soil mineral nitrogen using meta-analytic techniques.

Nitrogen enters the soil system by atmospheric deposition, fertilization, animal excretions, as dead organic matter, or via microbial fixation of atmospheric nitrogen by symbiotic or free-living microorganisms. Plants that do not have a symbiotic relationship with nitrogen-fixing microorganisms must take up nitrogen from the soil via their roots. During decomposition, nitrogen present in organic material is oxidized into inorganic compounds. The transformation of nitrogen is a dynamic process where specialized microorganisms gain energy by the conversion of nitrogen compounds, and - depending on the energy source used - excrete excess ammonium, nitrite or nitrate. These compounds do not remain long in the soil, but are rapidly transferred to other nitrogen pools, i.e., they are used as energy source by other groups of microorganisms, assimilated by plants, immobilized in microbial biomass, or lost to the groundwater or atmosphere by leaching and volatilization. Although plants are able to take up both inorganic and organic nitrogen, such as amino acids (Paungfoo-Lonhienne et al. 2012), the contribution of organic nitrogen to the nitrogen cycle is usually not acknowledged (Farzadfar et al. 2021). As the uptake of amino acids seems to play a minor role for plant nutrition in many ecosystems (Kuzyakov & Xu 2013) and the quantitative role of organic nitrogen uptake by plants is still unknown (Farzadfar et al. 2021), we focus on inorganic soil nitrogen in this study.

Earthworms modify physical and chemical soil properties by burrowing, casting and mixing litter and soil, thus altering the habitat structure and nutrient availability. Soils inhabited by soil-dwelling earthworms are characterized by a higher porosity, more water-stable aggregates, increased water-holding capacity and water infiltration rates compared to soils without or with only litter-dwelling species (Anderson 1988a). By their influence on the microhabitat, earthworms may change microbial community composition, biomass and activity and thus indirectly affect nitrogen cycling. In presence of earthworms, microbial communities switch to smaller but more active communities (McLean et al. 2006) and nitrogen-related microbial enzyme activity is increased (Zheng et al. 2018, Xue et al. 2022). Microbial activity is increased in fresh casts (Tiunov & Scheu 2000, Aira et al. 2005) and burrow walls during the first weeks after formation (Scheu 1987). Earthworm casts, especially fresh casts, contain higher amounts of nitrogen in comparison to bulk soil (Groenigen et al. 2019). Furthermore, earthworms can contribute to soil nitrogen pools by the excretion of urine, mainly in the form of ammonia and urea (Lang & Russell 2022), or mucus, a mixture of carbohydrates and proteins (Guhra et al. 2020, Shutenko et al. 2020). Overall, earthworm-derived nitrogen is estimated to make up a considerable portion of a plant's nitrogen requirements (Curry & Byrne 1992, Whalen et al. 2000).

The way earthworms affect their environment and soil microorganisms differs between ecological groups. Epigeic earthworms live in the litter layer and mostly feed on litter material. By fragmenting litter, they make it accessible to other organisms and thus play an important role in decomposition. Epigeic earthworms mix mineral and organic soil layers to a limited extent, but comminuted litter material and nutrients are moved down the soil profile by water flows. They therefore can be expected to affect soil nitrogen in the organic layer and uppermost soil mineral layer only. Endogeic earthworms inhabit the upper mineral soil horizons where they feed on organic material in the soil. They produce non-permanent horizontal burrows which are refilled by casts, thus changing physical and chemical soil properties such as porosity or aggregate structure (Anderson 1988b). Endogeic earthworms burrow only little into the lower mineral soil layer (Marhan & Scheu 2006), their effect on soil nitrogen is thus expected to be mainly restricted to the topsoil. Anecic earthworms live in permanent vertical burrows, but feed on litter from the soil surface which they pull into their burrows. Thereby, they provide channels for water infiltration and soil aeration, contribute to the mixing of soil layers and the decomposition of organic material, and redistribute nutrients across soil layers. As the influence of anecic earthworms on the soil properties has been found to decrease with distance from the burrow (Tiunov & Scheu 1999, Don et al. 2008), the impact of anecic earthworms on soil nitrogen is expected to be concentrated to their burrows and the directly surrounding soil.

Aside from ecological groups, the impact of earthworms on microorganisms and soil nitrogen may be affected by soil characteristics, climatic conditions and resource quality and quantity. For instance, the impact of earthworms on microbial community structure was found to depend on the type of plant litter that was applied (Zheng et al. 2018). Dehydrogenase activity as an indicator for microbial activity is decreased in sandy and clay-loam soils when earthworms are present, whereas no effect of earthworms was found in clay soils (Caravaca & Roldan 2003). Earthworms increase soil microbial biomass and respiration in soils with low carbon content, but reduce them in soils with high carbon content (McLean et al. 2006). Also, earthworms cease to actively influence soil microorganisms under unfavorable climatic conditions such as extreme temperatures or low soil moisture, as earthworms reduce their feeding activities, migrate to more favorable areas in deeper soil layers, become quiescent or enter into diapause (Singh et al. 2019).

It is evident that earthworms play a crucial role in nitrogen cycling. Recent meta-analyses have shown that earthworm invasion into previously earthworm-free environments results in increased nitrate contents in the mineral soil (Ferlian et al. 2020), that soil nitrogen is significantly increased in earthworm casts in comparison to bulk soil (Groenigen et al. 2019) and that earthworms increase N_2O emissions (Lubbers et al. 2013). There is also evidence that soil ammonium and nitrate as well as nitrogen assimilation by plants are increased by earthworms (Xue et al. 2022). However, the presence of plants can also cause changes in chemical, physical and biological soil properties, and a quantitative synthesis that focuses on earthworm effects in bulk soil while excluding the influence of plants on soil nitrogen and incorporating the controlling influence of further biological and soil-related factors is lacking. To fill this gap, we conducted a meta-analysis of earthworm effects on soil mineral nitrogen in the form of ammonium (NH_4^+) and nitrate (NO_3^-), as well as total mineral nitrogen (ammonium + nitrate). Our main objectives were to (1) estimate the impact of earthworm activity on the various nitrogen compounds; (2) assess whether the direction and magnitude of earthworm effects depend on ecological (life-form) groups, earthworm biomass or abundance; and (3) test whether effect sizes were modulated by soil characteristics or experimental design. Based on our analysis, we furthermore aimed to identify knowledge gaps that should be addressed in future research for a better understanding of earthworm effects on nitrogen cycling.

2. Methods

2.1 Data search and selection

To create a database of earthworm effects on soil nitrogen, we performed a literature search of peer-reviewed publications that reported results on the effect of earthworms on soil ammonium, soil nitrate or total mineral nitrogen (the sum of NH_4^+ and NO_3^- pools in the soil; hereafter: N_{min}) published until March 2021. The search was conducted using the Web of Science research database within papers published between 1980 and 2021 using the keywords: (earthworm* OR oligochaet* OR lumbricid*) AND (‘N minerali$ation’ OR ‘nitrogen minerali$ation’ OR ‘mineral N’ OR ‘mineral nitrogen’ OR nitrification OR nitrogen NEAR/2 soil OR ammoni* NEAR/2 soil OR nitrate NEAR/2 soil OR ‘inorganic nitrogen’ OR ‘inorganic N’) within the topics search. This search returned 559 studies (Fig. 1). We furthermore included studies found by searching bibliographies of reviews and articles. After a first scan of article titles and abstracts to remove spurious hits, the remaining articles were screened for their suitability for a meta-analysis based on the following criteria: (1) at least one of the following nitrogen compounds were measured in soil samples using standard chemical methods: NH_4^+, NO_3^- or N_{min}; (2) there were no plants in the experiment, in order to isolate the impact of earthworms on soil nitrogen from plant effects; (3) no potentially toxic amendments, such as sewage sludge, were used; (4) mineral soils were used (with or without a litter layer, but excluding organic soils, e.g. peat). All included studies reported results for earthworm and control (without earthworms) treatments in which all factors (experimental setup, soil properties) were the same. We excluded articles that used litterbag experiments or where nitrogen leaching was measured. Articles on vermicomposting or comparing soil nitrogen in earthworm casts or burrows with bulk soil were also excluded. Two review papers were excluded and sixteen articles could not be obtained in full text either digitally or in print. In three cases, more precise or additional data was provided by the authors. The final number of articles suitable for the meta-analysis was 24, and the number of effect sizes provided by those articles was 411 (Tab. 1, database available at the BonaRes Data Repository, https://doi.org/10.20387/bonares-phyx-qj16.

2.2 Data extraction and preparation

Separate datasets were prepared for NH_4^+, NO_3^- and N_{min}. For each observation we extracted the means, number of replicates and standard deviation (SD) or standard error for control and earthworm treatment. Standard errors were transformed to standard deviation values. When data were reported in figures and not published in texts or tables, we estimated values using the image analysis software ImageJ (Rasband 1997). In cases where articles reported data on both ammonium and nitrate but not on N_{min}, we calculated N_{min} by summing up NH_4^+ and NO_3^-. Missing SD values were imputed by using the average coefficient of variation across all observations, estimated separately for NH_4^+, NO_3^- and N_{min}.

Several potential controlling factors (moderators) that may explain the variation in effect sizes were included in our meta-analysis (see Tab. S1 for an overview on data availability). For each observation, information on soils (texture, soil organic carbon (SOC), total initial soil nitrogen, soil C:N ratio, litter C:N ratio, initial N-compound content), organisms (species, ecological group, biomass and abundance) and experimental details (continent, study type (i.e. laboratory or field experiment), experimental duration, experimental temperature, sampling depth) were noted. Soil textures were categorized as clays (sandy clay, silty clay, clay), loams (sandy loam, loam, silt, silt loam, silty clay loam, sandy clay loam, clay loam) and sands (sand, loamy sand), as reported in the studies or estimated from the particle size distribution using a soil texture calculator (USDA 2019). Earthworms were categorized into three ecological groups (epigeic, anecic, endogeic; the intermediate group of epi-endogeic earthworms was assigned as epigeic) based on author statements and the World Earthworm Database Drilobase (http://taxo.drilobase.org). Experiments that included more than one ecological group were assigned as 'mixed'. The experimental duration was categorized as days (up to 3 days), week (> 3 to 7 days), month (> 1 week to 1 month), up to three months (> 1 month to 3 months) and longer than three months (> 3 months). These time spans were chosen as earthworms were found to increase soil ammonium within some days (Abail & Whalen 2019), but no impact was found in experiments lasting several weeks or months (Jun-Zhu et al. 2012, Amosse et al. 2015). In contrast, regarding soil nitrate and total mineral nitrogen, short-term experiments showed no earthworm effects, but increased concentrations were reported in experiments lasting at least one month (McColl et al. 1982, Jun-Zhu et al. 2012, Amosse et al. 2015). Experimental temperature was only noted in cases where temperature was held constant during the experiment.

2.3 Statistical analyses

All statistical analyses were performed using the statistical software R 4.0.3 (R Core Team 2021). We calculated effect sizes for earthworm impacts on soil nitrogen using the natural logarithm of the response

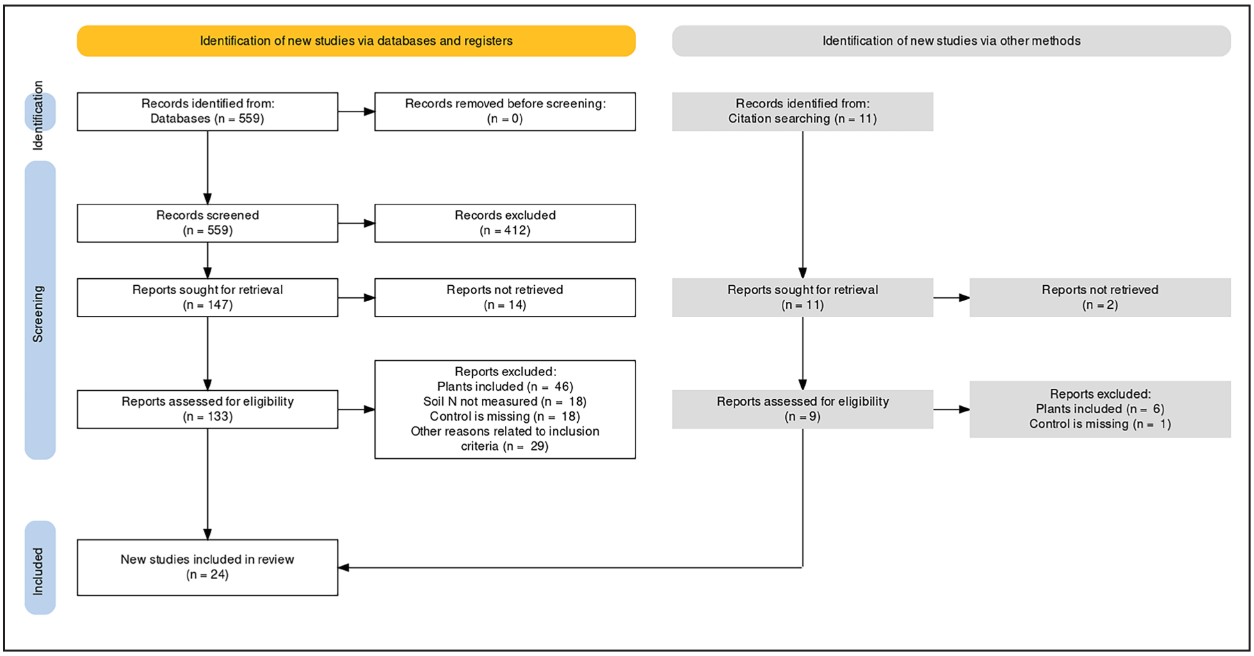

Figure 1. PRISMA flow diagram for the systematic literature search, created with the PRISMA2020 flow diagram R package (Haddaway et al. 2022). n refers to the number of research articles.

Table 1. Studies included in the meta-analysis and their characteristics. n = number of effect sizes calculated for each study. L/F = Laboratory experiment (L) or field experiment (F).

Reference	n	L/F	Continent	Taxon	Ecological group	N compound
Abail & Whalen 2019	12	L	North America	*Aporrectodea turgida* (Eisen, 1873)[a]	endogeic	NH_4^+, NO_3^-, N_{min}
Adejuyigbe et al. 2006	6	L + F	Africa	*Hyperiodrilus* sp. Beddard, 1891	epigeic	NH_4^+, NO_3^-, N_{min}
Amosse et al. 2015	36	L	Europe	*Aporrectodea rosea* (Savigny, 1826) , *Allolobophora chlorotica* (Savigny, 1826)	endogeic	NH_4^+, NO_3^-, N_{min}
Araujo et al. 2004	24	L	South America	*Rhinodrilus contortus* Černosvitov, 1938, *Pontoscolex corethrurus* (Muller, 1856)	endogeic	NH_4^+, NO_3^-, N_{min}
Bityutskii et al. 2012	6	L	Europe	*Aporrectodea caliginosa* (Savigny, 1826)	endogeic	NH_4^+, NO_3^-, N_{min}
Bohlen & Edwards 1995	90	L	North America	*Lumbricus terrestris* Linnaeus, 1758, *Aporrectodea tuberculata* (Eisen, 1874)[a]	anecic, endogeic	NH_4^+, NO_3^-, N_{min}
Brown et al. 1998	6	L	North America	*Lumbricus rubellus* Hoffmeister, 1843	epigeic	N_{min}
Devliegher & Verstraete 1997	1	L	Europe	*Lumbricus terrestris* Linnaeus, 1758	anecic	NO_3^-
Greiner et al. 2012	3	L	North America	*Amynthas hilgendorfi* (Michaelsen, 1892)[b], *Lumbricus rubellus* Hoffmeister, 1843	epigeic	N_{min}
Hamamoto & Uchida 2019	6	L	Asia	*Metaphire hilgendorfi* (Michaelsen, 1892)[b], *Eisenia fetida* (Savigny, 1826)	epigeic	NH_4^+, NO_3^-, N_{min}
Helling & Larink 1998	3	L	Europe	*Lumbricus terrestris* Linnaeus, 1758	anecic	N_{min}
Jana et al. 2010	3	L	Europe	*Aporrectodea caliginosa* (Savigny, 1826)	endogeic	NH_4^+, NO_3^-, N_{min}
Jun-Zhu et al. 2012	30	L	Asia	*Eisenia fetida* (Savigny, 1826)	epigeic	NH_4^+, NO_3^-, N_{min}
Makoto et al. 2016	12	L	Asia	*Aporrectodea rosea* (Savigny, 1826)	endogeic	NH_4^+, NO_3^-, N_{min}
Marhan et al. 2015	12	L	Europe	*Aporrectodea caliginosa* (Savigny, 1826)	endogeic	NH_4^+, NO_3^-, N_{min}
McColl et al. 1982	27	L	Australia	*Allolobophora caliginosa* (Savigny, 1826)	endogeic	NH_4^+, NO_3^-, N_{min}
Qiu & Turner 2017	18	L	North America	*Amynthas agrestis* (Goto & Hatai, 1899), *Amynthas tokioensis* (Beddard, 1892)[e]	epigeic	NH_4^+, NO_3^-, N_{min}
Ruz-Jerez et al. 1992	12	L	Australia	*Lumbricus rubellus* Hoffmeister, 1843, *Eisenia fetida* (Savigny, 1826)	epigeic	NH_4^+, NO_3^-, N_{min}
Sandor & Schrader 2012	3	L	unknown	*Lumbricus terrestris* Linnaeus, 1758, *Aporrectodea caliginosa* (Savigny, 1826)	anecic/ endogeic	NO_3^-
Sierra et al. 2014	8	L	North America	*Polypheretima elongata* (Perrier, 1872)[d], *Eudrilus eugeniae* (Kinberg, 1866), *Pontoscolex corethrurus* (Muller, 1856)	anecic, epigeic, endogeic	N_{min}
Willems et al. 1996	36	L	North America	*Lumbricus terrestris* Linnaeus, 1758, *Aporrectodea tuberculata* (Eisen, 1874)[a]	anecic, endogeic	NH_4^+, NO_3^-, N_{min}
Wu et al. 2015	36	L	Asia	*Eisenia fetida* (Savigny, 1826)	epigeic	NH_4^+, NO_3^-, N_{min}
Wu et al. 2021	15	L	Asia	*Metaphire guillelmi* (Michaelsen, 1895)[c]	endogeic	NH_4^+, NO_3^-, N_{min}
Zheng et al. 2018	6	L	Asia	*Metaphire guillelmi* (Michaelsen, 1895)[c]	endogeic	NO_3^-

valid names based on gbif.org: [a] *Aporrectodea caliginosa*; [b] *Perichaeta hilgendorfi* Michaelsen, 1892; [c] *Perichaeta guillelmi* Michaelsen, 1895; [d] *Perichaeta elongata* Perrier, 1872; [e] *Perichaeta tokioensis* Beddard, 1892 ratio, *lnR = ln(E/C),* where *E* and *C* are the mean nitrogen contents in the earthworm treatment and the control treatment without earthworms, respectively (Hedges et al. 1999). Positive values signify a higher nitrogen content in the earthworm treatment compared to the control, and negative values a lower nitrogen content in the earthworm treatment. As some measurements of nitrogen compounds were zero, we added 0.001 to all observations before calculating the response ratio to avoid invalid values in the determination of effect sizes. To ease interpretation, the results are reported as percentage change in the main text (% change = (exp(lnR)-1)*100).

Some studies contributed to the analysis with multiple observations (measurements at multiple time points or for various sampling depths) or different earthworm treatments were compared to the same control. We accounted for the arising non-independence of such data by using robust variance estimation following the steps: 1) Effect sizes were calculated using the *escalc* function in the package *metafor* (version 2.5-86, Viechtbauer 2010). 2) A variance-covariance matrix was imputed using the *impute_covariance_matrix* function from the *clubSandwich* package (version 0.5.2, Pustejovsky 2020). As sampling *covariances* were not available from publications, a correlation of r = 0.5 was assumed between multiple outcomes from the same study when experimental units contributed more than one observation or they shared a control treatment. 3) Multilevel meta-analytic models were fitted using the *rma.mv* function in *metafor*, with a random intercept term where 'ID' (a unique value for each row in the dataset) was nested within 'Reference' (a unique value for each citation included in the dataset). 4) A cluster-robust approach with a small-sample correction based on a Satterthwaite approximation was used to compute robust tests and confidence intervals (functions *coef_test* and *conf_int* in the package *clubSandwich* with the CR2 variance estimator). This accounts for the misspecification of the variance-covariance matrix in step (1).

As most moderator variables were not available for all observations, separate models were fit for each moderator using the data subsets containing the corresponding moderator data. Data paucity did not allow testing for interactive effects of moderators. Effect sizes were considered significant when their 95% confidence intervals did not overlap with zero. In cases where Satterthwaite degrees of freedom were less than four, the p-value associated with the test is likely to under-estimate the type I error (Tanner-Smith et al. 2016). In those cases, covariates involved in the test may be skewed or imbalanced and therefore we used a threshold of $p < 0.01$ (Tanner-Smith et al., 2016). To ensure that the variance and correlation components were identifiable in the models, profile likelihood plots were used. The robustness of our results was checked by running a sensitivity analysis. For this, we repeated the analyses with a low (r = 0.1) or a high (r = 0.9) covariance and compared our results.

3. Results

Our final dataset contained 411 observations, thereof 127 observations for NH_4^+, 137 for NO_3^- and 147 for N_{min}. Observations were available for 16 different earthworm species, with experiments containing one (251 observations) or two species (160 observations). In all experiments that contained epigeic earthworms, a litter layer was supplied.

No statistical difference was found between different study types (i.e. laboratory or field study; NH_4^+: p = 0.85; NO_3^-: p = 0.77; Nmin: p = 0.85) and continents (NH_4^+: p = 0.47; NO_3^-: p = 0.86; Nmin: p = 0.87). Earthworm presence significantly increased the amount of NO_3^- (+ 88%) and N_{min} (+ 63%), whereas the mean effect on NH_4^+ content (+ 41%) was not significantly different from zero (Fig. 2, Tab. S2). The effect of earthworms on NH_4^+, NO_3^- and N_{min} was not significantly affected by abundance (NH_4^+: p = 0.33; NO_3^-: p = 0.08; N_{min}: p = 0.41) or biomass (NH_4^+: p = 0.33; NO_3^-: p = 0.34; N_{min}: p = 0.93).

Observations for endogeic and epigeic earthworms or mixed ecological groups were available for all three nitrogen compounds. We did not find a significant effect on NH_4^+ for any ecological group (Tab. 2). Endogeic and epigeic earthworms significantly increased the amount

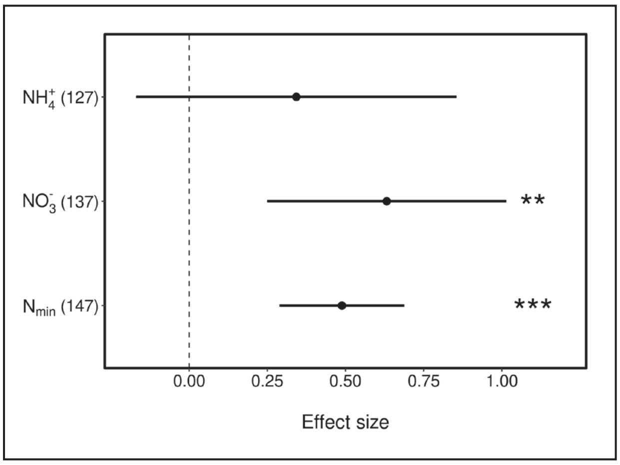

Figure 2. Mean effect sizes (logarithm of the response ratio) of earthworm presence on NH_4^+, NO_3^- and N_{min} with 95% confidence intervals. The number of observations is shown in parentheses. Significance levels are indicated by asterisks.

of NO_3^- (endogeic: + 140 %; epigeic: + 70 %) and N_{min} (endogeic: + 83 %; epigeic: + 54 %). A sufficient number of observations for anecic earthworms was only available for N_{min}. Taking the low degrees of freedom into account, no significant effect was found. All experiments on mixed ecological groups used a combination of endogeic and anecic earthworms. Interestingly, no significant effect of mixed ecological groups was found despite the positive effect of endogeic earthworms on NO_3^- and N_{min}. Whether ecological groups affect soil nitrogen in different soil depths could not be investigated as soil samples were usually mixed over the whole sampling depth.

3.1 Influence of soils and experimental conditions

The majority of experiments used loamy soils, only few experiments were conducted in clays and none in sands. Earthworm presence did not affect NH_4^+, NO_3^- or N_{min} in clay soils (Tab. 3). In loamy soils, earthworms had a significant effect on NO_3^- (+ 106 %) and N_{min} (+ 62 %), but no significant effect on NH_4^+. Taking the degrees of freedom into account, no significant moderating effect was found for initial values of SOC (NH_4^+: $p = 0.11$; NO_3^-: $p = 0.03$, d.f. = 1.03; N_{min}: $p = 0.06$), total soil nitrogen

Table 2. Results for the earthworm effect on NH_4^+, NO_3^- and N_{min} differentiated by ecological groups. **n** – number of observations; **LRR** – log-response ratio; **95 % CI** – 95 % confidence intervals; **SE** – standard error; **d.f.** – degrees of freedom. Significant effect sizes are shown in bold, results that are considered not significant due to low degrees of freedom (see methods for explanation) are shown in cursive.

Ecological group	n	LRR	95 % CI	SE	d.f.	P-value
NH_4^+						
anecic	0	-	-	-	-	-
endogeic	49	0.193	-0.638, 1.024	0.357	7.56	0.605
epigeic	36	0.219	-0.412, 0.851	0.242	4.75	0.408
mixed	42	1.128	-12.252, 14.508	1.055	1	0.479
NO_3^-						
anecic	1	0.168	-	-	-	-
endogeic	**55**	**0.877**	**0.101, 1.653**	**0.343**	**8.93**	**0.031 ***
epigeic	**36**	**0.531**	**0.0243 1.039**	**0.198**	**4.98**	**0.044 ***
mixed	*45*	*0.189*	*0.054, 0.323*	*0.031*	2	*0.026 **
N_{min}						
anecic	5	0.592	0.081, 1.33	0.05	1.1	0.043 *
endogeic	**51**	**0.604**	**0.197, 1.012**	**0.18**	**8.81**	**0.009 ****
epigeic	**49**	**0.431**	**0.257, 0.606**	**0.075**	**7.65**	**<0.001 *****
mixed	42	0.185	-0.538, 0.908	0.057	1	0.19

Note: The large CIs for NH_4^+ and mixed ecological groups result from robust variance estimation with skewed data.

Table 3. Results for the earthworm effect on NH_4^+, NO_3^- and N_{min} differentiated by soil textures. **n** – number of observations; **LRR** – log-response ratio; **95 % CI** – 95% confidence intervals; **SE** – standard error; **d.f.** – degrees of freedom. Significant effect sizes are shown in bold.

Texture	n	LRR	95 % CI	SE	d.f.	P-value
NH_4^+						
Clays	13	0.298	-2.451, 3.05	0.216	1	0.4
Loams	94	0.217	-0.578, 1.01	0.349	8.56	0.549
NO_3^-						
Clays	13	0.303	-3.237, 3.84	0.279	1	0.474
Loams	**103**	**0.724**	**0.037, 1.41**	**0.312**	**11**	**0.041 ***
N_{min}						
Clays	21	0.314	-0.329, 0.958	0.149	2	0.171
Loams	**100**	**0.481**	**0.146, 0.815**	**0.15**	**9.81**	**0.01 ****

(NH_4^+: p = 0.16; NO_3^-: p = 0.89; N_{min}: p = 0.8), soil C:N ratio (NH_4^+: p = 0.24; NO_3^-: p = 0.19; N_{min}: p = 0.13) and litter C:N ratio (NH_4^+: p = 0.05, d.f. = 2.92; NO_3^-: p = 0.96; N_{min}: p = 0.09). We did also not find a moderating effect of the initial amount of NH_4^+ (p = 0.22), NO_3^- (p = 0.25) or N_{min} (p = 0.17), and experimental temperature (NH_4^+: p = 0.96; NO_3^-: p = 0.19; N_{min}: p = 0.32).

The impact of earthworm presence on NO_3^- and N_{min} did depend on experimental duration, whereas no significant moderating effect on NH_4^+ was found (Tab. 4). Earthworm presence did not affect NO_3^- and N_{min} in experiments lasting up to one week. When experiments lasted more than a week, earthworms significantly increased NO_3^- (1 week – 1 month: + 67 %; 1 – 3 months: + 89 %; > 3 months: + 155 %) and N_{min} (1 week – 1 month: +45 %; 1 – 3 months: + 73 %; > 3 months: + 99 %), with effects becoming progressively larger with time.

3.2 Sensitivity analysis

To check the robustness of our results, we ran a sensitivity analysis where we set the correlation between dependent outcomes to 0.1 or 0.9. The sensitivity analysis shows that our results are consistent across varying correlations (Tab. S3), with the exemption of the moderating effect of experimental duration. Here, we found a significant effect of earthworm presence on N_{min} already in experiments lasting between 3 and 7 days when we assumed a high correlation (r = 0.9), whereas no significant effect was found at lower correlations (r = 0.5 and r = 0.1).

4. Discussion

Our meta-analysis of earthworm effects on mineral nitrogen content in the bulk soil reveals that (1) NO_3^- and N_{min} are increased by earthworms; (2) the impact of earthworms on soil nitrogen depends on a species' ecological life form; and (3) the magnitude and significance of earthworm effects depend on soil texture and experimental duration. The exact mechanisms behind the earthworm effects cannot be disentangled in this framework, but comparison with known factors of the nitrogen cycle and individual study results allow suggestions for mechanistic explanations.

Table 4. Results for the earthworm effect on NH_4^+, NO_3^- and N_{min} differentiated by time categories. **n** – number of observations; **LRR** – log-response ratio; **95% CI** – 95% confidence intervals; **SE** – standard error; **d.f.** – degrees of freedom. Significant effect sizes are shown in bold.

Duration	n	LRR	95 % CI	SE	d.f.	P-value
NH_4^+						
≤ 3 Days	7	-0.134	-1.594, 1.327	0.363	2.15	0.746
> 3 Days – 1 Week	15	-0.243	-1.357, 0.871	0.437	5.12	0.601
> 1 Week – 1 Month	44	0.247	-0.471, 0.966	0.322	9.95	0.461
> 1 Month - ≤ 3 months	30	0.698	-0.464, 1.859	0.514	9.11	0.208
> 3 months	31	0.368	-0.347, 1.083	0.258	4.01	0.227
NO_3^-						
≤ 3 Days	7	0.409	-0.307, 1.124	0.202	2.54	0.153
> 3 Days – 1 Week	15	0.416	-0.065, 0.896	0.203	6.96	0.08
> 1 Week – 1 Month	**44**	**0.515**	**0.146, 0.884**	**0.17**	**12.76**	**0.01 ****
> 1 Month - ≤ 3 months	**40**	**0.639**	**0.189, 1.09**	**0.21**	**14.09**	**0.009 ****
> 3 months	**31**	**0.936**	**0.193, 1.678**	**0.273**	**4.24**	**0.024 ***
N_{min}						
≤ 3 Days	7	0.178	-0.202, 0.557	0.101	2.33	0.201
> 3 Days – 1 Week	15	0.311	-0.014, 0.637	0.126	4.97	0.057
> 1 Week – 1 Month	**44**	**0.373**	**0.079, 0.667**	**0.133**	**10.6**	**0.018 ***
> 1 Month - ≤ 3 months	**43**	**0.55**	**0.263, 0.838**	**0.134**	**13.6**	**0.001 ****
> 3 months	**35**	**0.689**	**0.393, 0.984**	**0.118**	**5.42**	**0.002 ****

4.1 Effects of earthworm presence on soil mineral nitrogen

Our results of earthworm presence increasing total mineral nitrogen and nitrate, but not ammonium, are in line with the study by Ferlian et al. (2020) on the impact of earthworm invasion. In contrast to our results, the meta-analysis by Xue et al. (2022), which included plants in the experiments, reports a significant increase of not only nitrate but also ammonium. Earthworms and plants can have opposite effects on soil nitrogen compounds (James & Seastedt 1986, Jana et al. 2010), and effects of plants and earthworms together may not be complementary. For instance, NH_4^+ was found to decrease in presence of earthworms and to increase in presence of plants, but presence of both earthworms and plants resulted in an even lower NH_4^+ content than in the earthworm treatment (Jana et al. 2010). Also, earthworms increased NO_3^- contents, but this effect was lower in presence of plants (Jana et al. 2010). In order to isolate the impact of earthworms and as a first step towards a better understanding of earthworm effects, we only used observations from experiments without plants. Thereby, we excluded confounding effects of nitrogen uptake by plants, which can be increased in presence of earthworms (Brown et al. 1998, Adejuyigbe et al. 2006) and can mask earthworm effects on soil nitrogen (Fraser et al. 2003). Based on our results, we conclude that the positive effect of earthworms on mineral nitrogen is mainly through their impact on soil nitrate.

Higher earthworm biomass and/or abundance was expected to result in stronger earthworm effects on soil nitrogen (Sheehan et al. 2006, Jun-Zhu et al. 2012) due to higher overall activity. In contrast, we did not find a biomass- or abundance-dependent impact of earthworms in our meta-analysis. However, this non-significant result cannot necessarily be interpreted as a lack of earthworm abundance or biomass effects. Earthworm abundance and biomass in many experiments largely exceeded numbers that would be expected in natural habitats. Natural earthworm abundance typically ranges between 5 and 150 individuals per m^2, and biomass typically ranges between 1 to 150 g per m^2 (Phillips et al. 2019), but our data span abundances from 22 to 1624 individuals per m^2, and biomasses from 34 to 7305 g per m^2. We did not exclude experiments with extremely high abundances and biomasses as those stem from experiments using anecic earthworms which usually use mesocosms with a high depth and a small diameter. A possible explanation for the lack of abundance and/or biomass effects is that, in the experiments analyzed here, the higher densities and biomass may have caused a 'saturation' of earthworm effects. Our analysis is furthermore based on the initial abundance and biomass as most publications did not report values for the time of soil sampling. We can therefore not rule out that abundance and/or biomass had changed until the time of sampling. As earthworm growth and reproduction is influenced by the quality and availability of food resources (Edwards & Bohlen 1996), abundance and/or biomass may have changed during the experiment depending on the experimental conditions. Also, earthworm biomass in experiments with high initial earthworm abundance may have decreased during the experiment due to mortality or high earthworm densities negatively affecting growth rates and reproduction (Butt et al. 1994, Uvarov 2009).

4.2 Effects of ecological groups

The different life form types (ecological groups) of earthworms affected soil N differently, where endogeic and epigeic earthworms significantly increased NO_3^- and N_{min} and no significant effect of anecic earthworms was found. However, as only a low number of observations was available for the impact of anecic earthworms on N_{min}, further analyses with better replication would provide a more robust estimation of their impact on N_{min}.

Species from different ecological life-form types may affect soil nitrogen differently due to their divergent influence on the microhabitat and microbial communities. A recent meta-analysis has shown that microbial community composition varies in the presence of different earthworm ecological types, often with increased relative abundance of Proteobacteria in the presence of endogeic and epigeic earthworms, whereas anecic earthworms apparently facilitate Acidobacteria (Medina-Sauza et al. 2019). Proteobacteria play an important role in the nitrogen cycle as they include nitrogen-fixing bacteria (e.g. *Azotobacter*), ammonia-oxidizing bacteria (e.g. *Nitrosomonas* or *Nitrosococcus*) and nitrite-oxidizing bacteria (e.g. *Nitrobacter*). The role of Acidobacteria is less clear (Kalam et al. 2020), but several members are able to reduce nitrate, whereas there is no evidence of their involvement in nitrogen fixation, nitrification or denitrification (Kielak et al. 2016). This may explain the strong positive impact of endogeic and epigeic earthworms on NO_3^-, whereas anecic earthworms could be expected to increase ammonia via nitrate reduction. Unfortunately, we could only analyze the effect of anecic earthworms on N_{min} due to a lack of data for NH_4^+ and NO_3^-. Further research is needed to assess whether the influence of anecic earthworms on N_{min} is due to their influence on NH_4^+.

Interestingly, no effect of earthworm presence on soil nitrogen was found for species combinations from mixed ecological groups (i.e. anecic and endogeic earthworms), suggesting that the presence of anecic earthworms lessened the positive effect of endogeic earthworms on NO_3^- and N_{min}. This could be caused by anecic earthworms and their influence on the microbial community, as anecic earthworms are reported to become the dominant group, whereas endogeic earthworm densities decreased in mixed communities (Sheehan et al. 2007). Observations of other combinations of ecological groups and the combination of all ecological groups are lacking. This represents a knowledge gap that should be addressed in future research.

Endogeic and epigeic earthworms were expected to affect soil nitrogen in the top soil and the uppermost soil organic layer, respectively. However, in the analysed experiments, soil samples were usually mixed over the whole sampling depth. Therefore, we could not investigate whether ecological groups affect soil nitrogen differently at different soil depths.

4.3. Influence of soil, temperature and experimental parameters

We further attempted to explain variability in our results by analyzing the controlling influence of soils, climatic conditions and experimental parameters. Our meta-analysis shows that NO_3^- and N_{min} are especially increased by earthworms in longer lasting experiments (more than one week). This agrees with individual studies reporting stronger effects of earthworms on NO_3^- and N_{min} in longer-duration experiments (McColl et al. 1982, Jun-Zhu et al. 2012, Amosse et al. 2015). Several studies also reported earthworms increasing NH_4^+ within days (Abail & Whalen 2019) or weeks (Willems et al. 1996, Jun-Zhu et al. 2012, Amosse et al. 2015, Wu et al. 2015), but not for longer periods of time, i.e. longer than one month (Jun-Zhu et al. 2012, Amosse et al. 2015). In contrast, our meta-analysis could not show any effect of experimental duration on the influence of earthworms on NH_4^+. This suggests a rapid transformation of NH_4^+ into NO_3^- by nitrifying microorganisms, regardless of the presence or absence of earthworms.

The impact of earthworm presence on soil nitrogen was not affected by temperature, although earthworm activity is generally influenced by soil temperature, with extreme temperatures resulting in reduced earthworm activity (Singh et al. 2019). This non-significant result may be caused by the more favorable temperatures used in experiments: the temperature range for the observations in our dataset is between 10 and 27°C and temperature extremes were not covered. Also, earthworms may cause higher N_2O emission at elevated temperatures (Marhan et al. 2015). Thus, earthworm activity may cause a stronger increase of soil nitrogen at higher temperatures, but also a larger loss of nitrogen from the soil system by increased gaseous emission.

Our results further show that earthworms have a positive impact on NO_3^- and N_{min} in loamy soils, but not in clay soils. Loamy soils provide good conditions for earthworm population and biomass growth (Turbé et al. 2010). Also, the impact of earthworms on microbial activity is stronger in sandy or loamy soils (Caravaca & Roldan 2003). However, only a low number of observations was available for clay soils and no data at all for sandy soils. The moderating influence of soil texture on earthworm effects could thus not be comprehensively investigated and further research is needed.

Carbon availability is a major driver of earthworm activity and nitrogen mineralization (Sierra et al. 2014, Creamer et al. 2016) and the addition of organic carbon may increase microbial nitrogen immobilization (Cao et al. 2021). However, nitrogen conversion processes may be driven by the chemical quality of the carbon pool (labile, intermediate, recalcitrant) instead of the C:N ratio of soil or litter (Cao et al. 2021). Unfortunately, the analyzed studies did not report the chemical quality of carbon pools. We did not find significant moderating effects for SOC, total soil nitrogen, soil C:N ratio, litter C:N ratio and the initial amount of NH_4^+, NO_3^- or N_{min}. Data coverage for some of these parameters was low, for instance only a third of the observations report data on SOC or the soil C:N ratio and around half of the observations report the litter C:N ratio. In our analysis, the moderating effect of SOC on NO_3^- and of litter C:N ratio on NH_4^+ was considered non-significant due to low degrees of freedom. We thus call for more studies taking these factors into account to assess the importance of SOC and soil or litter C:N ratio for the influence of earthworms on soil nitrogen.

5. Conclusions and outlook

Our study provides quantitative evidence for a significant increase of total soil mineral nitrogen due to earthworm activity. This effect seems to be mainly caused by increased soil nitrate, whereas the impact of earthworms on soil ammonium is less pronounced. Moreover, the impact of earthworms differs among ecological life-form types. We speculate that the different impact of ecological groups is caused by a divergent influence on microbial community composition. The study furthermore reveals important knowledge gaps regarding earthworm effects on soil nitrogen that should be addressed in future research. First, research is strongly biased towards simple systems containing only up to two earthworm species or ecological groups, whereas observations of more complex earthworm communities are missing. Second, the moderating influence of some factors could not be comprehensively investigated as replication within factor categories was low or no data were available (i.e. anecic earthworms, sandy soils, clay soils). Third, data paucity also prohibited investigating interactive influences of moderating factors (i.e. soil properties, temperature and experimental characteristics) on the impact of earthworms in general and of different ecological groups. Nonetheless, our study highlights the importance of earthworms for soil nitrogen cycling and strengthens the call for models of soil nutrient cycling to integrate soil faunal effects.

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8. Author contributions

All authors contributed to the conception and design of the study. BL collected the data and reviewed the literature. BL and BBC analysed the data. All authors interpreted the results. BL wrote the first draft of the manuscript and all authors provided their comments and edited subsequent versions of the manuscript. All authors read and approved the final manuscript.

9. Additional information

Dataset: The dataset used in the current study is provided via the BonaRes Data Repository: https://doi.org/10.20387/bonares-phyx-qj16.

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Supplementary Information

Table S1. Overview on data availability with ranges (continuous factors) or subgroups covered (categorial factors). The number of observations for each moderator is shown in parentheses.

	NH_4^+	**NO_3^-**	**N_{min}**
Moderator	**Subgroups/ranges**	**Subgroups/ranges**	**Subgroups/ranges**
Texture	Clays (13), loams (94)	Clays (13), loams (103)	Clays (21), loams (100)
SOC (g kg^{-1})	12.1 – 40 (33)	9 – 40 (43)	12.1 – 40 (33)
Total soil N (g kg^{-1})	1.1 – 3.6 (96)	0.7 – 3.6 (105)	0.8 – 3.9 (106)
Soil C:N ratio	8.04 – 22 (47)	8.04 – 22 (56)	8.04– 22 (47)
Litter C:N ratio	8 – 48 (65)	8 - 150.8 (73)	8 – 48 (67)
Initial N-compound value (mg kg^{-1})	0.06 – 40.14 (77)	0 – 112.28 (80)	0.09 – 115.17 (85)
Species	*Allolobophora chlorotica* (Savigny, 1826) (4), *Aporrectodea caliginosa* (Savigny, 1826) (20), *Aporrectodea rosea* (Savigny, 1826) (8), *Eisenia fetida* (Savigny, 1826) (25), *Hyperiodrilus* sp. Beddard, 1891 (2), *Lumbricus rubellus* Hoffmeister, 1843 (2), *Perichaeta guillelmi* Michaelsen, 1895 (5), *Perichaeta hilgendorfi* Michaelsen, 1892 (1), *Pontoscolex corethrurus* (Muller, 1856) (4), *Rhinodrilus contortus* Černosvitov, 1938 (4), mixed species (52)	*Allolobophora chlorotica* (Savigny, 1826) (4), *Aporrectodea caliginosa* (Savigny, 1826) (20), *Aporrectodea rosea* (Savigny, 1826) (8), *Eisenia fetida* (Savigny, 1826) (25), *Hyperiodrilus* sp. Beddard, 1891 (2), *Lumbricus rubellus* Hoffmeister, 1843 (2), *Lumbricus terrestris* Linnaeus, 1758 (1), *Perichaeta guillelmi* Michaelsen, 1895 (11), *Perichaeta hilgendorfi* Michaelsen, 1892 (1), *Pontoscolex corethrurus* (Muller, 1856) (4), *Rhinodrilus contortus* Černosvitov, 1938 (4), mixed species (55)	*Allolobophora chlorotica* (Savigny, 1826) (4), *Aporrectodea caliginosa* (Savigny, 1826) (20), *Aporrectodea rosea* (Savigny, 1826) (8), *Eisenia fetida* (Savigny, 1826) (25), *Eudrilus eugeniae (4), Hyperiodrilus* sp. Beddard, 1891 (2), *Lumbricus rubellus* Hoffmeister, 1843 (9), *Lumbricus terrestris* Linnaeus, 1758 (3), *Perichaeta elongata* Perrier, 1872 (2), *Perichaeta guillelmi* Michaelsen, 1895 (5), *Perichaeta hilgendorfi* Michaelsen, 1892 (2), *Pontoscolex corethrurus* (Muller, 1856) (6), *Rhinodrilus contortus* Černosvitov, 1938 (4), mixed species (53)
Ecological group	Endogeic (49), epigeic (36), mixed (42)	Anecic (1), endogeic (55), epigeic (36), mixed (45)	Anecic (5), endogeic (51), epigeic (49), mixed (42)
Abundance (Ind m^{-2})	22 – 1052 (123)	22 - 1052 (133)	22 – 1624 (143)
Biomass (g m^{-2})	39 – 653 (64)	40 – 1671 (74)	34 – 7305 (82)
Duration	Days (7), week (15), < 1 month (44), < 3 months (30), > 3 months (31)	Days (7), week (15), < 1 month (44), < 3 months (40), > 3 months (31)	Days (7), week (15), < 1 month (44), < 3 months (43), > 3 months (35)
Temperature (°C)	15 – 25 (68)	15 – 25 (71)	10 – 27 (88)

Table S2. Detailed results for the general earthworm effect on NH_4^+, NO_3^- and N_{min} without moderators. **n** – number of observations; **LRR** – log-response ratio; **95% CI** – 95% confidence intervals; **SE** – standard error; **d.f.** – degrees of freedom. Significant effect sizes are shown in bold.

N-compound	n	LRR	95% CI	SE	d.f.	P-value
NH_4^+	127	0.343	-0.17; 0.856	0.241	15.2	0.175
NO_3^-	**137**	**0.632**	**0.249; 1.01**	**0.183**	**18.8**	**0.003 ****
N_{min}	**147**	**0.489**	**0.289; 0.689**	**0.096**	**19.5**	**<0.001 *****

Table S3. Results for the sensitivity analysis with a variance-covariance matrix that assumed a correlation of 0.5 (main analysis), 0.9 or 0.1 between dependent outcomes. Shown are the significance levels for the general earthworm effect and moderator variables. For categorical moderators, results are shown for each category separately. Asterisks indicate levels of significance (* P = 0.05, ** P = 0.01, *** P < 0.001). n.s. – not significant. Significance levels in parentheses denote results which are not considered significant based on low degrees of freedom.

	NH_4^+			NO_3^-			N_{min}		
Moderator	**r = 0.5**	**r = 0.9**	**r = 0.1**	**r = 0.5**	**r = 0.9**	**r = 0.1**	**r = 0.5**	**r = 0.9**	**r = 0.1**
General effect	n.s.	n.s.	n.s.	**	**	**	***	***	***
Ecological group									
Anecic	NA	NA	NA	NA	NA	NA	(*)	(*)	(*)
Endogeic	n.s.	n.s.	n.s.	*	*	*	**	**	**
Epigeic	n.s.	n.s.	n.s.	*	*	*	***	***	***
Mixed	n.s.	n.s.	n.s.	(*)	(*)	(*)	n.s.	n.s.	n.s.
Biomass	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Abundance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Texture									
Clays	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Loams	n.s.	n.s.	n.s.	*	*	*	**	*	**
Initial soil N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Initial NH4+ / NO3- / Nmin	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil C:N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Litter C:N	(*)	(*)	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
SOC	n.s.	n.s.	n.s.	(*)	(*)	(*)	n.s.	n.s.	n.s.
Temperature	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Duration									
Days	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
< 1 Week	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
< 1 Month	n.s.	n.s.	n.s.	**	*	*	*	*	*
1 – 3 months	n.s.	n.s.	n.s.	**	**	**	**	**	**
> 3 months	n.s.	n.s.	n.s.	*	*	*	**	**	**