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Excretion of nitrogenous waste by soil fauna and assessment of the contribution to soil nitrogen pools

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Abstract

The provisioning of nitrogen for plant growth is a key function of soils. Soil fauna primarily affect nitrogen mineralization through their interactions with microorganisms, but the excretion of feces and nitrogenous waste products can also supply plants with a considerable amount of their nitrogen requirements. The influence of soil fauna on soil nitrogen is rarely considered in agricultural soils. High amounts of mineral fertilizers are often applied, which are likely to be leached or denitrified from the soil if the amount of plant-available nitrogen exceeds crop requirements. This has profound consequences for the environment. Thus, we require a better understanding of the role of soil fauna in nutrient cycling to improve fertilizer management and agricultural sustainability. To this end, we review the current state of knowledge on the excretion of nitrogenous waste by soil fauna, focusing on earthworms, enchytraeids, nematodes, springtails, mites, isopods and myriapods. This includes an overview on excretory organs and products, a summary of quantitative measurements of nitrogen excretion and the factors that influence nitrogen excretion. Furthermore, we assess the contribution of soil faunal nitrogen excretion to nitrogen pools in agricultural fields based on mean nitrogen excretion rates and common soil invertebrate biomasses. Our results show that earthworms and nematodes are most likely to contribute agronomically-relevant quantities of nitrogen via excretion. Despite the very preliminary nature of our calculations, our results stress the importance of a better understanding of the role of soil fauna in nitrogen cycling in order to reduce soil-nitrogen losses and improve agricultural sustainability.

Keywords Nitrogen cycle | ammonia | urea | sustainability | agricultural management

1. Introduction

One of the key functions of soils is the provision of nutrients for plant growth. Nitrogen is a key nutritional element for all life forms as it is an essential element in amino acids, nucleic acids, chitin and proteins. The main actors for the transformation processes within the nitrogen cycle are microorganisms, but nitrogen mineralization is also affected by interactions between soil fauna and microorganisms. Furthermore, soil fauna can directly affect soil nitrogen pools by the excretion of feces (i.e. the part of the food consumed which is not assimilated) and nitrogenous waste products (i.e. the products of nitrogen transformation by the organism). In agricultural and natural ecosystems, around 30% of nitrogen mineralization are assigned to soil faunal activity (Neher & Barbercheck 1998). Aboveground plant biomass in agroecosystems was found to increase by 23% due to the presence of earthworms, most likely due to increased nitrogen mineralization (van Groenigen et al. 2014). Although a large part of nitrogen mineralization is driven by faunal-microbial interactions, the amount of nitrogen excreted from soil fauna can also be considerable, supplying plants with a substantial amount of their nitrogen requirements (Curry & Byrne 1992, Whalen et al. 2000, Schmidt & Curry 2001).

Nitrogenous waste in the form of ammonia is produced during the catabolism of proteins and amino acids and

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must be excreted to avoid toxic accumulation in body tissues. Ammonia is the most efficient nitrogenous waste product in energetic terms, but a high amount of water is required to dilute ammonia to concentrations below toxic levels. In aquatic organisms, ammonia is the major nitrogenous waste product as it can diffuse across membranes in its unprotonated form and is quickly washed away as it is soluble in water (Wright 1995). Terrestrial organisms would have to produce large quantities of urine to excrete ammonia, which means a high loss of water for the organism. To conserve water, terrestrial animals are generally assumed to excrete urea or purines (uric acid, guanine) at higher energetical costs, as urea excretion requires 10 times and purine excretion 50 times less water than ammonia excretion (Wright 1995). Soils are a heterogenous habitat that consists of the soil matrix (i.e. soil particles) and the pore system that is filled with soil water or air (i.e. the soil atmosphere). The water-filled pore space is inhabited by aquatic soil organisms such as nematodes, and the air-filled pore space is inhabited by non-aquatic soil faunal groups such as springtails or mites. Importantly, the soil atmosphere is saturated with water vapor, and soil animals are adapted to and depend on high moisture conditions (Bayley & Holmstrup 1999).

Despite the impact of soil faunal activity on nitrogen cycling, the quantity of nutrients in agricultural soils is usually adjusted by fertilizer application with high amounts of nitrogen being lost by leaching, volatilization or denitrification. This has profound consequences for the environment and human health, such as increased nitrate concentrations in the groundwater or increases of the greenhouse gas N_2O in the atmosphere. The common view for decades was that plants only take up inorganic nitrogen, but it is becoming progressively more apparent that uptake of organic nitrogen, including the nitrogenous waste product urea, is a common phenomenon (Schimel & Bennett 2004, Farzadfar et al. 2021). However, although excretion of nitrogenous waste by soil fauna is a source of nitrogen for plants, soil fauna are commonly not incorporated in models of nitrogen cycling and plant nutrition (Osler & Sommerkorn 2007).

In this review, we 1) aim to improve the understanding of the role of soil fauna in nitrogen cycling via excretion of nitrogenous waste, and 2) assess the contribution of soil faunal nitrogen excretion to nutrient pools in agricultural fields. In the first part of the review, the state of knowledge on the excretion of nitrogenous waste by soil invertebrate fauna is summarized, focusing on Oligochaeta (earthworms and enchytraeids), free-living nematodes, collembolans, soil mites, isopods and myriapods (Diplopoda and Chilopoda). For each taxonomic group, we present a short overview on the excretory organs and nitrogen compounds that are excreted. We do not aim to describe the mechanisms of nitrogen excretion in depth, as detailed overviews of the current knowledge are available elsewhere (e.g. Weihrauch & O'Donnell 2017). At the cellular level, nitrogenous waste is initially produced in the form of ammonium, which is mostly present in the ionic form (NH_4^+). In this review, the term ammonia refers to the sum of molecular ammonia (NH_3) and ammonium ions (NH_4^+). Second, we report species-specific quantitative measurements of nitrogen excretion. To facilitate the comparison of nitrogen excretion between organisms, all values are converted to µg N mg^{-1} day^{-1} by using the divisors 1.29, 1.22, 2.14 and 3 for the conversion of NH_4^+, NH_3, urea and uric acid, respectively. All measurements refer to the organism's wet weight in mg, the respective conversions are noted in each section. Third, we provide an overview of factors that may affect nitrogen excretion within each taxonomic group, such as temperature or feeding status. In the subsequent section, we assess the contribution of soil faunal nitrogen excretion to nutrient pools in agricultural fields. Nitrogen is not only a limiting factor for agricultural production, but is also lost from soils in large amounts. A better understanding of soil faunal contributions to nitrogen cycling may help to improve fertilizer recommendations and sustainability of agricultural systems. To this end, we estimate the amount of nitrogen excreted by soil fauna in agricultural fields based on the results of our review and average invertebrate biomass values found in the literature.

2. Excretion of nitrogenous waste

2.1 Earthworms

The main excretory organs in Oligochaeta are the metanephridia, which release urine through nephridiopores in the body wall. Nitrogen compounds are also found in the earthworm's cutaneous mucus, a mixture of proteins, carbohydrates, amino acids, peptidoglycans and fatty acids (Guhra et al. 2020, Shutenko et al. 2020) excreted by epidermal cells. The mucus facilitates respiration, prevents earthworms from desiccation and facilitates their movement through the soil (Edwards & Bohlen 1996), but does not seem to be involved in the elimination of nitrogenous waste (Abail & Whalen 2018). However, nitrogen excretion rates usually include both urine and mucus as a separation is difficult. In cases where fecal and urinary nitrogen were not separated, measurements of nitrogen excretion may include microbial nitrogen or nitrogen from non-assimilated food. A recent meta-analysis found the mineral nitrogen content in earthworm feces to be increased by 241 % compared to bulk soil (van

Groenigen et al. 2019). Inclusion of fecal nitrogen may thus strongly confound results, therefore experiments that include fecal nitrogen (e.g. Needham 1957) were not included in our summary of nitrogen excretion (Tab. 1).

Earthworms mainly excrete ammonia and urea (Edwards & Lofty 1972). A potential storage compound for nitrogen during starvation or dehydration is alanine, which has been found in high concentrations in estivating earthworms (Bayley et al. 2010). Quantitative measurements of nitrogen excretion are available for several species, and are mostly in the range from 0.004 to 0.7 µg N mg^{-1} day^{-1} (Tab. 1), with a mean nitrogen excretion across species of 0.677 µg N mg^{-1} day^{-1} . Exceptionally high values of urea excretion are reported by Babuthangadurai et al. (2014) for *Perionyx excavatus* Perrier, 1872. It is unclear whether this is a species effect or if the high nitrogen excretion is caused by the high experimental temperatures (32°C – 40°C).

Nitrogen excretion is reported to depend on the earthworm's feeding status, with earthworms switching from ammonotely (i.e. excretion of ammonia) to ureotely (i.e. excretion of urea) during starvation periods (Bishop & Campbell 1965, Cohen & Lewis 1949, Tillinghast et al. 1969). Earthworms may not respond immediately to changed food conditions, and the switching from ammonotely to ureotely in fasting earthworms can take some days (Cohen & Lewis 1949). In feeding earthworms, the type of food may affect nitrogen excretion. For plant litter with differing C:N ratio, no influence on excretion rates was found (Abail & Whalen 2019), but a lower excretion of nitrogen is reported when earthworms were fed carbohydrates in comparison to a protein diet (Tillinghast & Janson 1971). The influence of temperature on nitrogen excretion is unresolved. Whalen et al. (2000) did not find a significant effect of temperature on nitrogen excretion rates at 10°C and 18°C, whereas Tillinghast et al. (1969) report a higher urea excretion at 23°C in comparison to 8°C. Whalen et al. (2000) explain their results by the experimental temperatures being optimal for metabolic processes in the investigated earthworm species. However, it is important to notice that the experiments by Whalen et al. (2000) lasted two days whereas the experiments by Tillinghast et al. (1969) lasted several weeks, with increases in urea excretion becoming apparent after some days. Thus, the duration of the experiments may have been too short to detect a significant influence of temperature on nitrogen excretion.

In most experiments, earthworms were immersed in water, others used moistened filter paper as a substrate. Despite needing a certain amount of humidity to keep their skin moist, immersion in water for long periods is not the usual environmental condition for earthworms. The volume of water used was found to affect nitrogen excretion, particularly directly after a sudden change of water volume (Needham 1957) or at higher temperatures (Tillinghast et al. 1969). For instance, earthworms immersed in a larger volume of water excreted higher amounts of ammonia and lower amounts of urea at 23°C, but this effect was not found at 8°C (Tillinghast et al. 1969). Needham (1957) assumes that the switching to ureotely is a stress effect. Other stressors that may affect nitrogen excretion are heat stress (i.e. exceptionally high temperatures), acidic irritation or drought. For the earthworm *P. excavatus*, heat stress is reported to result in a decreased excretion of both ammonia and urea (Babuthangadurai et al. 2014). In comparison to non-stressed animals, acidic irritation stress resulted in an increased excretion of ammonia and a decreased excretion of urea (Babuthangadurai et al. 2014). In earthworms estivating due to drought conditions, a significant increase of the whole-body urea concentration is reported (Bayley et al. 2010). This may be a physiological adaptation to save water under severe dehydration, but could also be caused by the extended fasting period (Bayley et al. 2010). The different results suggest that the switching to ureotely is not a general stress effect, but may be specific to certain stressors, such as dehydration.

2.2 Enchytraeidae

Enchytraeidae belong to the Oligochaeta and, as with earthworms, the excretoric function is carried out by metanephridia which open by nephridiopores on the body surface. Enchytraeids are reported to be ammonotelic (Larsen et al. 2007, Maraldo et al. 2011). In contrast to the closely related earthworms, no urea excretion has been found so far (Larsen et al. 2007).

Quantitative measurements of nitrogen excretion are only available for two species, *Enchytraeus crypticus* Westheide & Graefe, 1992 and *Cognettia sphagnetorum* (Veydovsky, 1878) which were incubated on moist sand in these experiments (Tab. 2). Measurements range from 0.07 to 0.8 µg N mg^{-1} day^{-1} with a mean nitrogen excretion of 0.347 µg N mg^{-1} day^{-1}. For *C. sphagnetorum*, Maraldo et al. (2011) report a temperature dependence with higher nitrogen excretion at increased temperatures. There is no published information available on other factors that could influence nitrogen excretion.

2.3 Nematoda

Nematodes are a diverse group of animals that includes parasitic species and non-parasitic (free-living) species. In this review, we focus on terrestrial free-

Table 1. Nitrogen excretion by earthworm species at different temperatures. Given are mean values of nitrogen excretion per mg of earthworm wet weight and the range in parentheses when mean values were calculated from several treatments. Temp. = temperature; NA = not available

Species[a]	Temp. [°C]	Nitrogen compound	N excretion [$\mu g\ N\ mg^{-1}\ day^{-1}$][b]	References
Aporrectodea giardi (Ribaucourt, 1901); Lumbricidae	15	Ammonia	0.013	Gutierrez-Lopez et al. 2011
	20	Ammonia	0.006	Salmon 2001
Aporrectodea tuberculata (Eisen, 1874) (pre-clitellate); Lumbricidae	10	Total N[c]	0.625	Whalen et al. 2000
	18	Total N[c]	0.543	Whalen et al. 2000
Aporrectodea tuberculata (Eisen, 1874); Lumbricidae	10	Total N[c]	0.496	Whalen et al. 2000
	18	Total N[c]	0.744	Whalen et al. 2000
Aporrectodea turgida (Eisen, 1873); Lumbricidae	16	Total N[c]	0.609 (0.507 – 0.699)	Abail & Whalen 2019
Hormogaster elisae Alvarez, 1977; Hormogastridae	15	Ammonia	0.038	Gutierrez-Lopez et al. 2011
Lumbricus rubellus Hoffmeister, 1843; Lumbricidae	10	Total N[c]	0.543	Whalen et al. 2000
	18	Total N[c]	0.614	Whalen et al. 2000
Lumbricus terrestris Linnaeus,1758; Lumbricidae	8	Ammonia	0.019 (0.004 – 0.033)	Tillinghast et al. 1969
	8 – 12	Ammonia	0.004 (0.001 – 0.007)	Cohen & Lewis 1949
	13	Ammonia	0.012 (0.002 – 0.031)	Tillinghast & Janson 1971
	15	Ammonia	0.011 (0.001 – 0.034)	Tillinghast & Janson 1971
	23	Ammonia	0.022 (0 – 0.106)	Tillinghast 1967, Tillinghast et al. 1969
	8	Urea	0.016 (0.001 – 0.061)	Tillinghast et al. 1969
	8 – 12	Urea	0.023 (0 – 0.07)	Cohen & Lewis 1949
	13	Urea	0.049 (0.028 – 0.086)	Tillinghast & Janson 1971
	15	Urea	0.012 (0.001 – 0.089)	Tillinghast & Janson 1971
	23	Urea	0.089 (0 – 0.169)	Tillinghast 1967, Tillinghast et al. 1969
	10	Total N[c]	0.535	Whalen et al. 2000
	18	Total N[c]	0.532	Whalen et al. 2000
Lumbricus terrestris Linnaeus, 1758 (juvenile); Lumbricidae	10	Total N[c]	0.326	Whalen et al. 2000
	18	Total N[c]	0.278	Whalen et al. 2000
Perionyx excavatus Perrier, 1872; Megascolecidae	32	Ammonia	0.561 (0.452 – 0.669)	Babuthangadurai et al. 2014
	40	Ammonia	0.216	Babuthangadurai et al. 2014
	32	Urea	13.873 (11.473 – 16.273)	Babuthangadurai et al. 2014
	40	Urea	6.482	Babuthangadurai et al. 2014
Pheretima (Perichaeta) posthuma Vaillant, 1868; Megascolecidae	NA	Ammonia	0.043	Bahl 1945
	NA	Urea	0.015	Bahl 1945

[a] Species names are given as in the referenced publications; [b] mg of earthworm wet weight; [c] based on ^{15}N measurements

living nematodes which inhabit the water film in soil pores. Nematodes possess a nephridial system, whose complexity differs between nematode classes (Perry & Wright 1998), and seem to excrete ammonia at least in parts across the hypodermis (Adlimoghaddam et al. 2015, 2017). Nematodes are predominantly ammonotelic (Adlimoghaddam et al. 2015, 2017, Rothstein 1963, Wright 1975), but also excrete urea and uric acid to some extent (Adlimoghaddam et al. 2015, Perry & Wright 1998, Wright 1975). Furthermore, the excretion of aminoacyl-nitrogen, amino acids and peptides has been reported (Rothstein 1963, Thaden & Reis 2000, Thompson & Geary 2002, Wright 1975), but whether those compounds are excretory products is questioned by Wright (1998).

Quantitative measurements of nitrogen excretion are available for *Caenorhabditis elegans* (Maupas, 1900) and *Panagrellus redivivus* (Linnaeus, 1767), which are both bacterivorous species (Tab. 3). For nematodes belonging to other trophic groups, no measurements of nitrogen excretion were found. Mean rates of nitrogen excretion range from 0.03 to 1.79 µg N mg^{-1} day^{-1} with a mean nitrogen excretion of 0.602 µg N mg^{-1} day^{-1}. The excretion of nitrogen was not affected by nematode population density (Wright 1975). For *C. elegans,* an influence of the animals' feeding state on ammonia excretion is reported, with excretion in starving animals decreasing by 72 % in comparison to fed animals (Adlimoghaddam et al. 2015). Experiments using *P. redivivus* report an influence of the medium's volume, with decreased ammonia excretion when the medium's volume was increased (Wright 1975). In contrast, neither starvation nor the medium's volume were found to affect urea excretion (Adlimoghaddam et al. 2015, Wright 1975). In terms of environmental influences, ammonia excretion is reported to depend on pH, with a higher excretion in acidic medium and a lower excretion in alkaline medium (Adlimoghaddam et al. 2015). Ammonia excretion furthermore seems to be affected by the ammonia concentration in the medium as nematodes exposed to elevated sublethal ammonia levels showed ammonia excretion rates that were increased two- to threefold in comparison to control levels (Adlimoghaddam et al. 2015).

2.4 Collembola

The main excretory organs in Collembola are the labial nephridia located in the head (Hopkin 1997). Urine excreted via the nephridia is transported along the ventral groove (linea ventralis) to the ventral tube, at whose apical end the exchange of fluids and salts takes place (Hopkin 1997). Additionally, the renewal of the midgut epithelium during moulting provides a way to excrete waste or toxic products (Fountain & Hopkin 2005). However, a cytochemical study of the concretions found in midgut cells did not provide clear evidence for the presence of uric acid or urates by Humbert (1978). Ammonia is assumed to be the main excretory product as urea was not detected in experiments (Larsen et al. 2007). Quantitative measurements are available for seven species, with

Table 2. Nitrogen excretion by enchytraeid species differentiated by experimental temperature. Given are mean values of nitrogen excretion per mg of enchytraeid wet weight. Temp. = temperature

Species	Temp. [°C]	Nitrogen compound	N excretion [µg N mg^{-1} day^{-1}][a]	References
Cognettia sphagnetorum (Veydovsky, 1878)[b]	5	Ammonia	0.074	Maraldo et al. 2011
	10	Ammonia	0.105	Maraldo et al. 2011
	15	Ammonia	0.287	Maraldo et al. 2011
	20	Ammonia	0.818	Maraldo et al. 2011
Enchytraeus crypticus Westheide & Graefe, 1992[b]	21	Ammonia	0.45	Larsen et al. 2007

[a] mg of enchytraeid wet weight; [b] conversion based on dry weight = 16.5 % of wet weight (Dunger & Fiedler 1997)

Table 3. Nitrogen excretion by nematodes. Given are mean values of nitrogen excretion per mg of nematode wet weight and the range in parentheses when mean values were calculated from several treatments. Temp. = temperature

Species	Temp. [°C]	Nitrogen compound	N excretion [µg N mg^{-1} day^{-1}][a]	References
Caenorhabditis elegans (Maupas, 1900)	22	Ammonia	0.727 (0.118 – 1.787)	Adlimoghaddam et al. 2015, 2017
	22	Urea	0.113 (0.18 – 0.301)	Adlimoghaddam et al. 2015
Panagrellus redivivus (Linnaeus, 1767)	23	Ammonia	0.994 (0.448 – 1.793)	Wright 1975
	23	Urea	0.17 (0.126 – 0.222)	Wright 1975
	23	Uric acid	0.042 (0.034 – 0.05)	Wright 1975

[a] mg of nematode wet weight

ammonia excretion ranging from 0.005 to 1.66 µg N mg^{-1} day^{-1} (Tab. 4) with a mean nitrogen excretion across species of 0.2 µg N mg^{-1} day^{-1}. Interestingly, ammonia excretion reported by Larsen et al. (2007) at 21°C for *Folsomia fimetaria* (Linnaeus, 1758)*, Protaphorura armata* (Tullberg, 1869) and *Sinella curviseta* Brook, 1882 is many times higher in comparison to results by Sjursen & Holmstrup (2004) for the respective species at 20°C. This discrepancy is assumed to be caused by a different metabolic activity due to the length of starvation prior and during the experiments (Larsen 2007).

Nitrogen excretion was not found to be affected by population density (Sjursen & Holmstrup 2004). Investigations of the influence of temperature on nitrogen excretion showed mixed results depending on species. For *F. fimetaria, Folsomia candida* Willem, 1902 and *S. curviseta,* ammonia excretion seems to increase with increasing temperature across a range from 5°C to 20°C (Sjursen & Holmstrup 2004). In contrast, ammonia excretion seems to slightly decrease with increasing temperature in *Hypogastrura assimilis* (Krausbauer, 1898), whereas no influence of temperature is apparent in *P. armata* (Sjursen & Holmstrup 2004).

2.5 Mites

Mites are a diverse subclass of the arachnida whose members can be encountered in aquatic, marine and terrestrial habitats. In this review, we focus on free-

Table 4. Nitrogen excretion by collembola species differentiated by experimental temperature. Given are mean values of nitrogen excretion per mg of collembola wet weight and the range in parentheses when mean values were calculated from several treatments. Temp. = temperature

Species	Temp. [°C]	Nitrogen compound	N excretion [µg N mg^{-1} day^{-1}]^{a}	References
Folsomia candida Willem, 1902^{b}	5	Ammonia	0.02	Sjursen & Holmstrup 2004
	10	Ammonia	0.033	Sjursen & Holmstrup 2004
	15	Ammonia	0.046	Sjursen & Holmstrup 2004
	20	Ammonia	0.038 (0.021 – 0.066)	Sjursen & Holmstrup 2004
Folsomia fimetaria (Linnaeus, 1758)^{b}	5	Ammonia	0.079	Sjursen & Holmstrup 2004
	10	Ammonia	0.105	Sjursen & Holmstrup 2004
	15	Ammonia	0.138	Sjursen & Holmstrup 2004
	20	Ammonia	0.201	Sjursen & Holmstrup 2004
	21	Ammonia	1.104	Larsen et al. 2007
Hypogastrura assimilis (Krausbauer, 1898)^{b}	5	Ammonia	0.08	Sjursen & Holmstrup 2004
	10	Ammonia	0.092	Sjursen & Holmstrup 2004
	15	Ammonia	0.085	Sjursen & Holmstrup 2004
	20	Ammonia	0.053	Sjursen & Holmstrup 2004
Mesaphorura macrochaeta Rusek, 1976^{b}	21	Ammonia	1.656	Larsen et al. 2007
Proisotoma minuta (Tullberg, 1871)^{b}	21	Ammonia	1.02	Larsen et al. 2007
Protaphorura armata (Tullberg, 1869)^{b}	5	Ammonia	0.02	Sjursen & Holmstrup 2004
	10	Ammonia	0.023	Sjursen & Holmstrup 2004
	15	Ammonia	0.026	Sjursen & Holmstrup 2004
	20	Ammonia	0.014	Sjursen & Holmstrup 2004
	21	Ammonia	0.33	Larsen et al. 2007
Sinella curviseta Brook, 1882^{b}	5	Ammonia	0.005	Sjursen & Holmstrup 2004
	10	Ammonia	0.023	Sjursen & Holmstrup 2004
	15	Ammonia	0.041	Sjursen & Holmstrup 2004
	20	Ammonia	0.045	Sjursen & Holmstrup 2004
	21	Ammonia	1.185	Larsen et al. 2007

[a] mg of collembola wet weight; [b] conversion based on dry weight = 30% of wet weight (Dunger & Fiedler 1997)

living terrestrial mites that are associated with soil or litter habitats. Soil mites show a high diversity with respect to their ecology and morphology and various excretory systems are described. Malpighian tubules are present in Anactinotrichida (= Parasitiformes) but are absent or small in Actinotrichida (= Acariformes) (Alberti et al. 2003). Coxal glands (nephridia) are described in all acarine orders (Krantz & Walter 2009), but their function as excretory organ may be modified or lost in taxa that possess malpighian tubules (Alberti et al. 1996). Furthermore, the postventricular midgut may function as an excretory organ (Alberti et al. 2003). Mites are assumed to excrete guanine and uric acid (Krantz & Walter 2009). However, the interpretation of white accumulations as guanine seems to be purely based on visual inspection and is questioned by Alberti et al. (2003), who assume that those granules are rather mineral deposits containing amorphous calcium, magnesium phosphates or potassium. In a morphological study of 48 species of oribatid mites, no guanine or urate crystals were found in the coxal glands or digestive tracts, suggesting that oribatids rather excrete nitrogenous waste in a soluble form (Woodring 1973). Excretion of nitrogenous waste in a soluble form is also reported for gamasid mites with ammonia as an excretory product (Larsen et al. 2007, Sjursen & Holmstrup 2004), whereas no excretion of urea could be detected (Larsen et al. 2007).

Mites are among those groups of soil animals for which quantitative measurements of nitrogen excretion are scarce. In fact, quantitative measurements could only be found for a single gamasid mite species (Tab. 5) and seem to be lacking for other groups of soil mites. Nitrogen excretion ranges from 0.03 to 0.18 µg N mg^{-1} day^{-1} with a mean nitrogen excretion of 0.11 µg N mg^{-1} day^{-1} (Larsen et al. 2007, Sjursen & Holmstrup 2004) and seems to decrease with increasing temperature (Sjursen & Holmstrup 2004). The amount of excreted nitrogen reported by Larsen (2007) at 21°C is much higher than that reported by Sjursen & Holmstrup (2004) at 20°C, supposedly caused by a different metabolic activity due to the length of starvation (Larsen 2007).

2.6 Isopoda

Woodlice, isopods of the suborder Oniscidea, are crustaceans which made the evolutionary transition to a partly or completely terrestrial life. This required morphological, ecological and behavioral adaptations of reproduction, respiration and excretion, as well as a protection to counter evaporative water loss (Hornung 2011). The transition to terrestrial life is generally assumed to be accompanied by ureotely and uricotely (i.e. excretion of uric acid), but terrestrial isopods have retained ammonotely with gaseous ammonia as the main excretory product (Carefoot 1993). Hoese (1981) proposed that ammonia is excreted by the maxillary glands (nephridia) into the water-conducting system, where it is transported to the ventral pleopods and is volatilized during the transport (Greenaway 1991). Kirby & Harbaugh (1974) identified both the telson and the head as sites of ammonia release, but the telson was found to play the major role. In accordance with Kirby & Harbaugh (1974), Wright & Odonnell (1993) found the pleon fluid to be more important for ammonia excretion as it showed a higher concentration of ammonia than the maxillary urine and the haemolymph. The importance of the maxillary glands for nitrogen excretion is thus questioned (Wright & O'Donnell 1993).

As a large amount of water is needed to dilute ammonia to subtoxic concentrations, isopods face the problem of concomitant water loss. A solution may be to excrete ammonia during periods of high relative air humidity or during phases of active water vapor absorption (Wright & Odonnell 1993). For instance, isopods may excrete ammonia during daytime when they are inactive and hide in moist places (Kirby & Harbaugh 1974, Wieser et al. 1969, Wieser & Schweizer 1970, Wright & Peña-Peralta 2005). This means that nitrogenous waste has to be stored in a non-toxic way until its release. As potential storage compounds, several amino acids such as glutamine, glutamate, glycine and arginine have been reported (Wright et al. 1994, 1996). Interestingly, diel patterns of ammonia excretion are reported for experiments where animals were exposed to constant light or darkness (Kirby & Harbaugh

Table 5. Nitrogen excretion by mites at different temperatures. Given are mean values of nitrogen excretion per mg of mite wet weight. Temp. = temperature

Species	Temp. [°C]	Nitrogen compound	N excretion [µg N mg^{-1} day^{-1}][a]	References
Hypoaspis aculeifer (Canestrini, 1884)[b]	5	Ammonia	0.148	Sjursen & Holmstrup 2004
	10	Ammonia	0.105	Sjursen & Holmstrup 2004
	15	Ammonia	0.093	Sjursen & Holmstrup 2004
	20	Ammonia	0.033	Sjursen & Holmstrup 2004
	21	Ammonia	0.176	Sjursen & Holmstrup 2004

[a] mg of mite wet weight; [b] conversion based on dry weight = 40 % of wet weight (Dunger & Fiedler 1997)

1974, Wieser et al. 1969, Wieser & Schweizer 1970), but not when animals were kept under natural diurnal photofluctuations (Wieser 1972, Wieser et al. 1969).

Quantitative measurements of ammonia excretion are available for several species and range from 0.02 to 1.2 µg N mg^{-1} day^{-1} with a mean excretion of 0.109 µg N mg^{-1} day^{-1} (Tab. 6). Ammonia excretion differs between species and seems to be higher for males than for females (Wieser 1972). Further influences on ammonia excretion are the feeding status of the animals and environmental temperature. Animals that are allowed to feed are reported to excrete less ammonia in comparison to starving animals (Wieser et al. 1969, Wieser & Schweizer 1970). Temperature affects ammonia excretion with a higher excretion at increased temperatures (Wieser 1972). There are, however, exceptions such as *Oniscus asellus* Linnaeus, 1758 where no influence of temperature was found (Wieser 1972). Furthermore, ammonia excretion was found to vary seasonally with higher excretion in spring and summer than in autumn or winter (Wieser et al. 1969, Wieser & Schweizer 1970). The cause for this pattern is unclear, but it is speculated to reflect seasonal changes in metabolic rate or dietary nitrogen (O'Donnell & Wright 1995).

2.7 Myriapoda: Diplopoda and Chilopoda

Myriapods possess malpighian tubules as an excretory and osmoregulatory system, which join the digestive tract between midgut and hindgut (Hopkin & Read 1992, Rosenberg et al. 2011). The nitrogenous waste products are mixed with the digested food in the hindgut and are excreted together as fecal pellets (Hopkin & Read 1992). Chilopods of the groups Scutigeromorpha and Lithobiomorpha additionally possess maxillary nephridia at their head, which are assumed to function as excretory organs (Hilken & Rosenberg 2006, Rosenberg et al. 2009). However, actual measurements of nitrogen excretion by the maxillary nephridia are lacking and their function as an excretory organ is only inferred from morphological descriptions (Rosenberg et al. 2011). Ammonia as well as uric acid have been reported as excretory products for myriapods (Bennett 1971, Bennett & Manton 1962, Hopkin & Read 1992, Rosenberg et al. 2011).

Quantitative measurements of nitrogen excretion are lacking as the nitrogen from metabolic breakdown products is excreted together with fecal nitrogen. Observations that include fecal material found a higher amount of ammonia in the fecal pellets in comparison

Table 6. Nitrogen excretion by Isopoda at different temperatures. Given are mean values of nitrogen excretion per mg of isopod wet weight and the range in parentheses when mean values were calculated from several treatments. Temp. = temperature; NA = not available

Species	Temp. [°C]	Nitrogen compound	N excretion [µg N mg^{-1} day^{-1}]^{a}	References
Armadillidum vulgare (Latreille, 1804)	20	Ammonia	0.049	Wieser 1972
	30	Ammonia	0.102 (0.086 – 0.118)	Wieser 1972
	NA	Ammonia	0.056 (0.019 – 0.073)	Wright & Peña- Peralta 2005
Cylisticus convexus (De Geer, 1778)	20	Ammonia	0.074 (0.057 – 0.09)	Wieser 1972
	30	Ammonia	0.184	Wieser 1972
Ligidium lapetum Mulaik & Mulaik, 1942	NA	Ammonia	0.042 (0.015 – 0.069)	Wright & Peña-Peralta 2005
Oniscus asellus Linnaeus, 1758	20	Ammonia	0.079 (0 – 0.356)	Wieser 1972, Wieser et al. 1969, Wieser & Schweizer 1970
	23	Ammonia	0.17 (0 – 1.2)	Wieser et al. 1969
	30	Ammonia	0.103	Wieser 1972
	NA	Ammonia	0.071	Hartenstein 1968
Porcellio pictus Brandt, 1833	20	Ammonia	0.04 (0.026 – 0.054)	Wieser 1972
	30	Ammonia	0.187 (0.078 – 0.296)	Wieser 1972
Porcellio scaber Latreille, 1804	20	Ammonia	0.148 (0.05 – 0.433)	Wieser 1972, Wieser et al. 1969, Wieser & Schweizer 1970
	30	Ammonia	0.155 (0.149 – 0.162)	Wieser 1972
	NA	Ammonia	0.115 (0 – 0.305)	Wright et al. 1994

[a] mg of isopod wet weight

to the ingested food (Bano et al. 1976, Bocock 1963). However, the increase of nitrogen contents in fecal pellets is in large parts assumed to be caused by the death and autolysis of microorganisms in the gut and not due to the excretion of nitrogenous waste by the myriapods (Bano et al. 1976, Bocock 1963).

2.8 Summary of soil faunal nitrogen excretion

Tab. 7 summarizes the taxonomic groups and their excretory organs, the excretory products, the number of species for which measurements are available, and investigated factors that potentially influence nitrogen excretion. Most quantitative measurements are available for earthworms with observations of nitrogen excretion across eight species. With regard to other taxonomic groups, our review reveals a lack of research across functional or trophic groups. For instance, despite their high functional diversity, quantitative measurements for nematodes and mites are only available for bacterivorous and predatory species, respectively. Furthermore, observations are only available for very few species within mites, nematodes and enchytraeids. For Myriapoda, observations are completely lacking as they usually excrete urine together with feces. Various potential influencing factors were investigated across taxonomic groups. The animal's feeding status as well as some environmental influences were found to affect the chemical composition (i.e. ammonia or urea) or the amount of nitrogen excretion. However, results were not always consistent across taxonomic groups or species. For instance, temperature is reported to affect nitrogen excretion in enchytraeids and mites, but within earthworms, springtails and isopods no clear effect was found or results differed between species. In this regard, it is important to notice that within enchytraeids and mites, observations of temperature effects are only available for a single species. The cause of species-specific temperature effects is so far unresolved. In some experiments, the temperature gradient may not have been sufficient to detect significant effects on nitrogen excretion, especially when experiments were conducted within the optimal temperature range for metabolic processes. Further uncertainty is added by the variation of the individual's sex or developmental stage within experiments. The latter issue could be addressed in future research by using age-synchronized populations.

Although terrestrial animals are generally considered to be ureotelic or uricotelic, most soil invertebrate fauna are ammonotelic. Some groups of soil organisms inhabit the water-filled pore space (nematodes) or are highly dependent on soil water and are inactive when soil moisture is too low (earthworms, enchytraeids). Therefore, it is not surprising that those groups are ammonotelic. The ammonotely of isopods is seen as a remnant of their evolution from aquatic environments (Weihrauch & O'Donnell 2017), and they mainly excrete nitrogenous waste when relative air humidity is high (Wright & Odonnell 1993). The main nitrogenous waste product for springtails, mites and myriapods also appears to be ammonia. This can be explained by soil animals resembling aquatic animals more than truly terrestrial animals, as they are adapted to a soil atmosphere with a relative humidity close to 100 % (Holmstrup 2014). There is a constant influx of water across the springtail integument due to the high permeability to water and the osmotic pressure of their body fluids (Bayley & Holmstrup 1999). A net efflux of water already occurs at a relative humidity of 99.4 % (Bayley & Holmstrup 1999), but soil arthropods have evolved various strategies such

Table 7. Overview of taxonomic groups and species, excretory organs and products, and potential influencing factors. Factors which were found to affect nitrogen excretion are marked in bold; factors were results are mixed are marked in italics. NA = no data available

Taxonomic group	Number of species with available data	Excretory organs	Primary excretory products	Investigated factors
Earthworms	8 species	Metanephridia	Ammonia, urea	**Feeding status, type of food,** *temperature*, **substrate/water volume, stressors (heat, acid)**
Enchytraeidae	2 species	Metanephridia	Ammonia	**Temperature**
Nematoda	2 species	Nephridia	Ammonia, urea, uric acid	Population density, **feeding status, medium volume, elevated ammonia levels, pH**
Collembola	7 species	Nephridia, probably moulting	Ammonia	Population density, *temperature*
Mites	1 species	Malpighian tubules, coxal glands, midgut	Ammonia	**temperature**
Isopoda	6 species	Nephridia/maxillary glands, pleon fluid	Ammonia	**Sex, feeding status**, *temperature*, daily/**seasonal periodicity**
Myriapoda	NA	Malpighian tubules, maxillary nephridia	Ammonia, uric acid	NA

as cuticular protection or behavioral responses to balance their water budget during dry conditions (Kaersgard et al. 2004). Another strategy found in springtails and oribatid mites is to minimize water loss at lower relative humidity by actively increasing body fluid osmolality (Bayley & Holmstrup 1999, Slotsbo et al. 2017, Kaersgaard et al. 2004). To avoid a lethal influx of water when relative humidity increases, springtails have to remove osmolytes from their body fluids (Bayley & Holmstrup 1999). Hence, the excretion of urine provides a way to maintain the water balance and remove excess water.

We cannot rule out the possibility that other products are excreted as only a low number of observations is available for soil faunal groups and only some experiments report the absence of other nitrogenous waste products. For instance, no urea excretion could be found for enchytraeidae, collembola or mites (Larsen et al. 2007), and no or only a low excretion of uric acid is reported for earthworms (Bahl 1945, Cohen & Lewis 1949). Thus, our results are highly important with regard to plant nutrition as soil fauna excrete nitrogen mainly in the form of nitrogen compounds that can be directly taken up by plant roots. This does not only apply to ammonia, but also to urea which can be utilized by at least some plants (Farzadfar et al. 2021).

Measurements of nitrogen excretion in soil animals involve some inherent (technical) challenges. First, the amount of nitrogen excreted by soil animals is small due to their small size. This problem can be solved by using a larger number of individuals in experiments, often necessitating that specimens from laboratory cultures are used to ensure that all individuals used are of the same species. Second, nitrogen is excreted into the soil environment and technically feasible methods are required to extract the excreted nitrogen from the soil. A common solution is to set up an experiment in an artificial environment to enable the collection of nitrogenous waste products, e.g. by using glass dishes without any medium, with moistened filter paper or nitrogen-free moist sand as substrate, or by running experiments in liquid medium (buffer solution or water) without any further substrate. This may affect nitrogen excretion as, for example, the medium's volume was found to affect nitrogen excretion in earthworms and nematodes.

3. Significance of soil-faunal nitrogen excretion for agriculture

Nitrogen is a limiting factor for agricultural production and high amounts of mineral fertilizers are applied worldwide. However, only around 35 % of the globally applied nitrogen is used by plants (Omara et al. 2019) whereas high amounts are lost by leaching, soil erosion and gaseous emissions. A higher use of synthetic fertilizers results in even lower nitrogen use efficiencies (Lassaletta et al. 2014). To improve fertilizer recommendations and increase the sustainability of agricultural systems, contributions of soil organisms to nitrogen cycling should be considered (Whalen & Hamel 2004). To this end, we assessed the annual amount of nitrogen excreted by soil fauna (kg ha^{-1} $year^{-1}$) based on mean nitrogen excretion within each taxonomic group and mean soil faunal biomasses in agricultural fields (Tab. 8).

Nitrogen excretion was calculated using the quantitative measurements compiled for the first section of this review. Due to the scarcity of data across species and potential influencing factors, we used the overall mean within each taxonomic group. In cases where more than one nitrogenous waste product was measured in the same experiment (e.g. ammonia and urea), the total amount of nitrogenous waste produced in the experiment was used to calculate the mean. The activity of soil fauna strongly depends on environmental conditions such as temperature and soil moisture (Edwards 2004). For

Table 8. Density and biomass data used for the calculation of nitrogenous waste excretion in agricultural fields. CVT = Conventional tillage, CST = Conservation tillage, NT = No-tillage

Taxonomic group	Mean wet weight [mg]	Mean nitrogen excretion [µg N mg^{-1} day^{-1}][a]	Density [Individuals m^{-2}]			Biomass [mg m^{-2}]		
			CVT	CST	NT	CVT	CST	NT
Lumbricidae		0.244				20192	47596	103846
Enchytraeidae	0.643[b]	0.347	2875	6122	960	287.5	612.2	96
Collembola	0.057[b]	0.2	12941	11078	5490	3105.8	2658.7	1317.6
Mites	0.04[b]	0.111	16667	11176	980	1666.7	1117.6	98
Nematodes	0.0007[c]	0.887		12.26 x 10^6			8582	

[a] mean value for all available observations, per mg of animal wet weight; [b] based on Dunger & Fiedler 1997; [c] based on van den Hoogen et al. 2019

instance, earthworms can reduce their activity, become quiescent or enter diapause under unfavorable climatic conditions. We accounted for phases of inactivity by estimating nitrogen excretion for various levels of activity (i.e. soil fauna was assumed to be active between 50 and 80 percent of the year).

Biomasses were extracted from a review on effects of different tillage systems (conventional tillage, conservation tillage, no-tillage) on soil faunal communities with a focus on German data (van Capelle et al. 2012). We thus only included earthworms belonging to the family Lumbricidae in our estimation, as the other families included in our review (Hormogastridae and Megascolecidae) do not occur in Germany. As biomass data were only available for earthworms, we estimated mean biomasses of springtails, mites and enchytraeids based on their densities and mean fresh weights (Dunger & Fiedler 1997, van den Hoogen et al. 2019). Nematode densities for the various tillage systems were given in individuals 100 g^{-1} soil (van Capelle et al. 2012). To achieve comparability with the other soil faunal groups, we calculated nitrogen excretion by nematodes based on a mean density of 12.26 x 10^6 individuals m^{-2} for wheat or barley cultivation regardless of the tillage system (Crotty et al. 2016).

The calculations of potential soil fauna-excreted nitrogen must be regarded as a first approximation as they are based on data for few species and we therefore could not account for population variability (in different soils, regions or crop types), their dynamics, or influencing factors. Nonetheless, the calculated direct nitrogen inputs show that earthworms and nematodes contribute a large amount of nitrogen to soils via excretion of nitrogenous waste. In contrast, the mesofauna's contribution appears to be of less magnitude due to their lower biomasses (Tab. 9). With regard to the different tillage systems, earthworm-derived nitrogen can contribute a remarkable amount to plant nitrogen supply especially in no-till agriculture where earthworm biomass is comparably high. These results are in line with the findings of some individual studies that calculated a direct contribution of earthworms to nitrogen pools between 3 and 36 kg N ha^{-1} $year^{1}$ (Marinissen & de Ruiter 1993, Curry et al. 1995). To complete the picture of the influence of soil fauna on nitrogen supply, other sources of faunal nitrogen input (e.g. via the death of individuals or from earthworm mucus) and indirect effects on nitrogen mineralization have to be taken into account. However, despite the very preliminary nature of these calculations, our estimations emphasize the impact of soil faunal groups on nitrogen pools and stress the importance of a better understanding of the role of soil fauna in nitrogen cycling for a more sustainable agricultural management.

4. Conclusions

Despite the assumption that terrestrial animals' waste nitrogen excretion is generally via ureotely and uricotely, most soil faunal groups are primarily ammonotelic. This is highly significant since this means that they excrete nitrogen in a form that can be taken up by plants without a further transformation. Mean nitrogen excretion rates across taxonomic groups range from 0.1 to 0.7 µg N mg^{-1} day^{-1} and are affected by various factors such as the

Table 9. Estimates of nitrogen excretion by soil fauna for different tillage systems and percentages of activity. CVT = conventional tillage, CST = Conservation tillage, NT = No-tillage

Taxonomic group	Tillage system	N excretion [kg ha^{-1} $year^{-1}$]			
		80% activity	70% activity	60% activity	50% activity
Lumbricidae	CVT	14.39	12.59	10.79	8.99
Enchytraeidae	CVT	1.87	1.64	1.4	1.17
Collembola	CVT	0.43	0.38	0.32	0.27
Mites	CVT	0.22	0.19	0.16	0.14
Lumbricidae	CST	33.91	29.67	25.43	21.19
Enchytraeidae	CST	3.99	3.49	2.99	2.5
Collembola	CST	0.37	0.32	0.28	0.23
Mites	CST	0.14	0.13	0.11	0.09
Lumbricidae	NT	73.99	64.74	55.49	46.24
Enchytraeidae	NT	0.63	0.55	0.47	0.39
Collembola	NT	0.18	0.16	0.14	0.11
Mites	NT	0.013	0.011	0.009	0.008
Nematodes	Not specified	22.23	19.45	16.67	13.89

state of feeding and environmental stressors. For some taxonomic groups, and especially for functional groups, research is scarce or lacking. Our first estimations of soil faunal nitrogen excretion in agricultural fields reveal that earthworms and nematodes can contribute large amounts of nitrogen to soil nitrogen pools. The estimated magnitude of earthworm-derived nitrogen is especially high in no-till agriculture due to their high biomass. This implies that the direct impact of soil fauna on nitrogen pools, as well as explicit soil-fauna conservation goals, should be taken into account in order to reduce soil-nitrogen losses and improve sustainability of agricultural management.

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6. Author contributions

BL and DJR conceived the study. BL collected the data and reviewed the literature. All authors interpreted the results and contributed to the manuscript.

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