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Nitrification inhibitors: biological and synthetic[1]

1 Measure definition

Nitrification inhibitors (NIs) are compounds that delay bacterial oxidation of NH4+ to NO3- (Nitrification) by depressing the enzymatic activities of nitrifiers (e.g. Nitrosomonas) in the soil (Subbarao et al. 2006). NIs were developed to prevent nitrate leaching by stopping bacteria in the soil from converting nitrogen from fertilisers or animal urine into nitrate. Inhibition of nitrification can improve the sustainable use of nitrogen by reducing nitrate leaching to groundwater (Qiao et al. 2015). Lower nitrate concentrations in soils also contribute to reduced nitrous oxide emissions.

Geographical and biophysical applicability

- **Suitability to different biophysical conditions:** They can be used in different cropping systems across various climatic regions (Subbarao et al. 2006). Because a wide geographical range of plant species possess nitrification inhibitory effect (Wang et al. 2021), BNIs can be locally applied in different geographical regions. SNIs are less effective in soils with heavy texture, high soil organic matter as this might cause sorption of the inhibiting compounds and affect its mobility (Subbarao et al. 2006). For example, in a plane loamy soil in Wisconsin, US, nitrap yearin completely inhibited nitrification in soils with 1 % SOM and at higher pH whereas this was not effective in soils with 5 % SOM (Hendrinkson and Keeney 1979). Also, in an arable soil in Germany, SNIs like DCD were found to perform better at reducing nitrate formation in sandy than in loam and clay soils (Barth et al. 2019). This is not surprising since their original application was to prevent nitrate leaching from sandy soils.

- **Suitability in EU/German conditions:** SNIs are widely used on conventional farms with livestock and/or biogas production, where ammonia-rich slurries prone to gases and dissolved nitrogen losses are regularly applied. They are also widely used by arable farms with light soils and urea-based fertilisation regimes. The further expansion of SNIs is limited because of the European and German goal to increase the share of organic agriculture to 30 % and SNIs are per definition not compliant with the EU organic regulation.

Nitrification inhibitors can be either biological (BNI) or synthetic (SNI)[2] (Coskun et al. 2017).

Subbarao et al. (2006) listed 64 synthetic compounds which have been proposed as SNI. Most of these SNIs inhibit the first enzymatic step of nitrification (inhibition of the ammonia oxidase enzyme AMO) (Ruser and Schulz 2015). Commercially and widely utilised SNIs are nitrap yearin, dicyandiamide (DCD) and 3,4-dimethylp yearazole phosphate (DMPP) (Ruser and Schulz 2015; Subbarao et al. 2006). Nitrap yearin and dicyandiamide (DCD) belong to a large extent to the inhibition group of Cu chelators and the same mechanism of inhibition is also assumed for DMPP

[1] This factsheet was developed as part of the research project "Naturbasierte Lösungen (NbS) im Klimaschutz: Marktanreize zur Förderung klimaschonender Bodennutzung" (FKZ 3721 42 502 0) and is also published as part of the Annex to the UBA report "Role of soils in climate change mitigation", see www.umweltbundesamt.de/publikationen/Role-of-soils-in-climate-change-mitigation. The factsheet was amended in December 2023. Specifically, on page 2 the first sentence in 2nd paragraph was changed from "Biological nitrification inhibitors (BNIs) are an alternative to SNIs" to "In addition to synthetic inhibitors, biological nitrification inhibitors (BNIs) can also be applied." In section 5 on implementation challenges, a new paragraph was included, starting with the sentence "Currently, the approval process for release of nitrification inhibitors to the market ….".

[2] There are also urea inhibitors (UI). SNIs and UIs are often grouped together as "inhibitors", however they are chemically different and have different modes of action. This factsheet focuses on SNI and BNI.

(Ruser and Schulz, 2015.), whereby a strict classification of SNIs in only one group of inhibitors is not possible. However, some SNIs also carry risks for soil health and biodiversity as they can be ecotoxic for terrestrial and aquatic organisms: in a study of two commercial NIs (Piadin and Vizura) and an active ingredient of another NI (dicyandiamide (DCD)), Piadin and Vizura showed ecotoxic effects in all experiments conducted (Kössler et al. 2019). Concerns have also been raised about risk to human health since the active ingredient, dicyandiamide (DCD), was found as a residue in milk (Ray et al. 2020). This underlines the importance of applying the precautionary principle and a comprehensive risk assessment.

In addition to synthetic inhibitors, biological nitrification inhibitors (BNIs) can also be applied. Some plant species have the natural ability to release compounds (from either their root, rhizosphere, tissue, litter or tissue extract) that suppresses the activity of nitrifiers (Subbarao et al. 2013a; Wang et al. 2021; Zhang et al. 2021). Examples of BNIs extracted from root tissues are linoleic acid and linolenic acid (Ma et al. 2021). Common temperate crops with BNI function are the pasture grass and some landraces of wheat (O'Sullivan et al. 2016). E.g., BNI levels in the rhizosphere of wheat landraces ranged from 25-45 % reduction in nitrification (O'Sullivan et al. 2016). Some BNIs secreting crops such as sorghum and Brachiaria grasses can be used as cover crops (Subbarao et al. 2013b).

The research on BNI is still in its infancy and more crop species or varieties may exudate compounds with BNI function that can be integrated in crop rotations. The extraction and technical production of BNI might then become feasible (Wang et al. 2021). Research and breeding for BNI expression of the relevant plant genes in the rhizosphere of important crops may also provide new management options for improving nitrogen efficiency in cropping systems (Zhang et al. 2021).

Information on the prevalence of use of SNIs and BNIs is not available.

1.1 Fit with NbS definition

Various plant species release molecules with different chemical properties as root exudates that regulate soil nitrification by blocking the enzymatic pathways of nitrifying bacteria, e.g. Nitrosomonas (Subbaroa et al. 2013). This suggests the alignment of BNIs with nature.

Exudates released from plant roots, i.e. BNIs, can be seen as an adaptive mechanism for the efficient conservation and use of nitrogen in natural ecosystems including agricultural systems where nitrogen is limiting (Subbarao et al. 2006; Qiao et al. 2015; Ma et al. 2021).

However, SNIs are synthetic chemicals applied as external inputs and potentially with negative side-effects, therefore their alignment with natural ecosystems is not given.

2 Mitigation Potential

2.1 Carbon sequestration

There is currently no available research on the effects of NIs on soil carbon sequestration rates and SOC stocks even within the EU.

2.2 Total climate impact

Total GHG balance:

Multiple studies have reported a reduction in N_2O emission rates by 18 to up to 92 % using different BNIs (Wang et al. 2021, Ruser and Schulz 2015). A 67-76 % reduction in N_2O emissions with the use of SNIs has been reported for arable soils in LA, US (Meng et al. 2021).

In a European grassland site in Wales, UK, with very high nitrification rates, Ma et al. (2021) reported an up to 93.5 % reduction in NO3- concentration with 1g linoleic acid per kg of soil which is a BNI found in Brachiaria spp (Subbarao et al. 2008). Studies on BNI on GHG emissions within European regions are currently still limited.

In a global meta-analysis using 62 studies, SNIs were found to decrease direct N_2O emissions by 39-48 % and NO3- leaching by 38-56 %, leading to a net reduction of 16.5 % of total nitrogen loss to the environment (Qiao et al. 2015).

However, some nitrification inhibitors carry the risk of higher ammonia (NH_3) emissions in some pedo-climatic conditions (Wang et al.2020; Qiao et al. 2015).

2.3 Limitations on the mitigation potential

The efficacy, synthesis and release of BNIs from plants as well as SNIs are highly variable and vary depending on the type of NIs released, the presence of NH4+, the abundance of the soil nitrifier population in the rhizosphere and the soil chemical and physical properties such as soil texture, organic matter content, pH, moisture and temperature, oxygen concentration (Coskun et al. 2017; Gopalakrishnan et al. 2009; Subbarao et al. 2013a; Subbarao et al. 2006).

For effectiveness, NI compounds must retain their persistence and bioactivity in the soil, thus the effectiveness of NIs in soils often depend on the length of time they can be persistent in soils (Subbarao et al. 2006). Loss of NIs by volatilisation, leaching and microbial turnover decrease their effectiveness in soils (Hendrinkson and Keeney 1979; Ruser and Schulz 2015).

Common SNIs like Nitrap yearin can be specific and effective on some bacterial groups (e.g. Nitrosomonas) over others (e.g. Nitrobacter) (Subbarao et al. 2006). Thus, the composition of the nitrifier community should be considered before adoption.

Despite laboratory-based evidence for the inhibitory activity on some microbial nitrifiers, not all BNIs released from plant roots will be effective in suppressing soil nitrification activity in the field (Lu et al. 2019). Some BNIs have been found to lose their activity in soils after 80 days (Subbarao et al. 2008). Due to this uncertainty, further field testing is required (Wang et al. 2021). Respective information reported for soils within Europe is also missing in the current literature.

3 Adaptation and co-benefits

- **Yields and efficiency:** There is some evidence that the use of SNIs in combination with split fertiliser application or no-till cultivation can lead to an increase of crop yields of up to 7 % (Del Grosso 2009), while increasing the nitrogen use efficiency (NUE) indicated by higher N uptake (Abolos et al. 2014).
- **Soil quality:** Soil acidification is one of the most common consequences of soil degradation caused by N overuse (Qiao et al. 2015). Reducing N overuse could therefore contribute to alleviating soil acidification. The long-term impact of SNIs on the soil microbiome is uncertain. Agrochemicals such as NIs may bear the risk of developing tolerant populations or

negative effects on non-target organisms. Hence further research is needed ideally by focussing on soils with a long-term history of NI application (Ruser and Schulz 2015).

- **Air pollution:** SNI application significantly decreases NO emissions by up to 38 % (Qiao 2015).
- **Water quality:** The use of SNIs and BNIs reduce the nitrification process and the risk of leaching of NO3- and therefore have the potential to improve the quality of waterbodies close to agricultural areas. The use of N fertiliser can lead to nitrification and leaching of NO3-. NO3- moves through the soil and potentially ends up in water bodies leading to euthrophication and health risks for aquatic organisms (Subbaro et al. 2006).
- **Human health:** The use of SNIs and BNIs reduces the nitrification process, thus leading to reduced nitrate leaching and reducing the risk of high nitrate concentrations in groundwater and therefore also the risk of nitrate consumption. Nitrate consumption can lead to human health risks through drinking contaminated water or consuming high nitrate containing vegetables ultimately leading to various kinds of human cancer, neural tube defects, diabetes and blue baby syndrome (Ahmed et al. 2017).
- **Economic benefits:** There is evidence that the economic benefit of reducing N's environmental impacts offsets the cost of SNI application with a potential increase of revenues for the farmers of up to 9 %, using a US maize farm as a case study (Qiao et al. 2015).
- **Energy saving:** The use of SNIs can ultimately result into saving energy inputs into agricultural systems due to the decreased amount of N fertiliser use. The process of producing synthetic N fertiliser requires a considerable amount of energy plus the energy spent for transport, application and incorporation (Subbaro et al. 2006).

4 Trade offs

- **Soil:** SNIs reduce the activity and abundance of target nitrifying bacteria (Nitrosomonas genera), but also shift the abundance of non-target bacteria. The negative effects of fertilisation on soil functionality are partially alleviated but the complexity of bacterial interaction networks can be reduced (Corrochano-Monsalve et al. 2021).
- **Disease** resistance **in crops:** SNIs can influence disease development and host resistance e.g. in corn, soybean and potato (Subbaro et al. 2006).
- **Water bodies:** There is research that indicates ecotoxic effects of SNIs on terrestrial and aquatic organisms (Kösler et al. 2019).
- **Air pollution:** There is a risk of higher ammonia (NH3) emissions with the use of some SNIs in some pedo-climatic conditions (Wang et al. 2020; Qiao et al. 2015).

5 Implementation challenges

There are several implementation challenges acting as a barrier for the uptake of SNIs and BNIs as practices by farmers, including uncertain effects of SNI and BNI usage under field conditions since these effects also depend strongly on weather conditions after application, application knowledge of farmers, additional cost and regulation/restrictions. SNIs are not allowed in organic agriculture. Beyond this, once the produce is approved, no further regulation of their use is set. Often, SNIs are already included in synthetic urea fertilisers.

Currently, the approval process for the release of nitrification inhibitors to the market (as per REACH regulation) does not investigate the possible impacts of NI on soil microbiome and its key functions to soil health. The only soil microbial ecotoxicology assessment required is the respiratory test to assess overall soil microbial activity. However, there are strong indications that NI impacts also on non-target organisms, those microbial groups who are not involved in the nitrification process. Therefore, future REACH registration mechanisms for agrochemicals with dedicated microbial knock-out traits need to consider a more comprehensive soil microbiome assessment. Soil ecological expertise from Julius-Kühn-Institut (JKI) and Thünen-Institut should be included for developing a state-of-the-art soil microbiome assessment protocol. On-going research projects such as NitriKlim (https://www.thuenen.de/de/institutsuebergreifende-projekte/nitriklim-bewertung-von-nitrifikationsinhibitoren-als-klimaschutzmassnahme) will further contribute to such concepts.

Given the unclear long-term impacts of synthetic NIs on soil biodiversity, precautionary principles should be applied. Until further clarity is available on long-term effects, the use of SNIs should be restricted.

The use of BNIs is still in its infancy, with limited knowledge on their NI specificity, pathways, locations, mechanisms of release and interactions with other BNIs and with other biotic and abiotic components of the soil matrix and the environment (Coskun et al. 2017).

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