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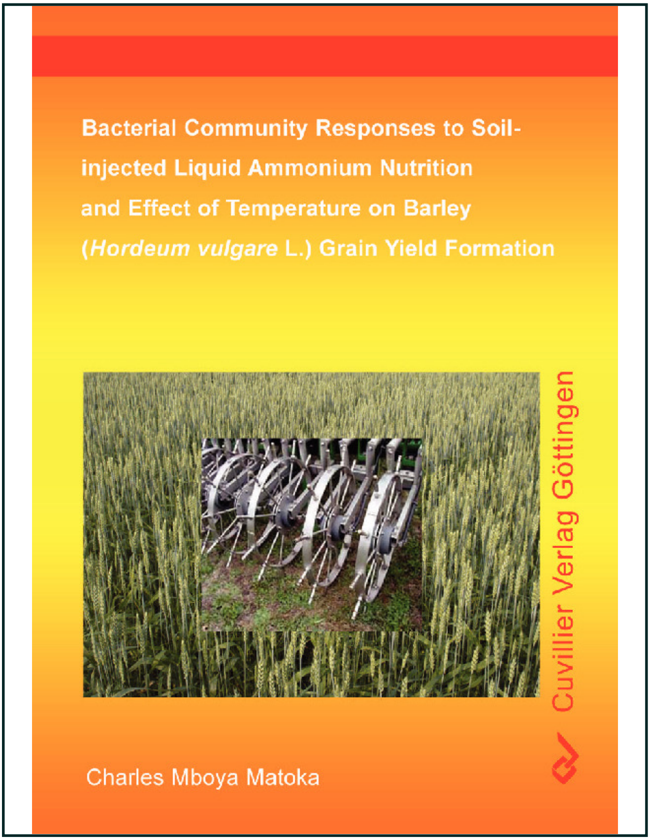
Bacterial community responses to Soil-injected liquid ammonium nutrition and effect of temperature on barley (Hordeum vulgare L.) grain yield formation



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1.0 INTRODUCTION

1.1 Need for improved fertilization methods

Nitrogen (N) is one of the nutrients required for plant growth since it forms an integral part of various vital compounds. Upon uptake, it is may be assimilated into plant tissues and organs. Normally, nitrogen accounts for about 2-5% of the total plant dry matter. For this reason, it is usually required in appreciably larger amounts than the other nutrients (Marschner, 1995). The use of commercial fertilizers, especially nitrogen aims at crop growth and yield improvement. However, losses associated with the currently employed conventional fertilization methods calls for the adoption of alternative methods or improvement of the existing ones. The application of liquid ammonium fertilizer is emerging as a noble fertilization technique because it mitigates nitrate associated losses.

1.2 Nitrogen forms taken up by crops

In soil, nitrogen occurs both as organic and inorganic compounds, with 95% or more being organic (Miller and Cramer, 2004). The apparent inadequacy of nitrogen availability for uptake by crops necessitates the application of commercial fertilizers and has made it a common agricultural practice. Generally, nitrate and ammonium are the main inorganic nitrogen sources taken up by roots of higher plants. Upon uptake, NO_3^- can be reduced either within the root or shoot and excess amounts can be stored in different plant tissues like vacuoles (Marschner, 1995). Since NH_4^+ is usually toxic to plants when taken up in large amounts, it is thus a prerequisite that crops to which it is exposed must be well adapted to either prefer or tolerate its effects (Gerendas et al., 1997). Crops fertilized by NH_4^+ should be capable of regulating both internal and external acidic pH conditions to avoid 'ammonia syndrome' resulting from ionic imbalances (Mehrer and Mohr, 1998). Because of its toxicity effects on crops, NH_4^+ is normally assimilated within the roots (Britto and Kronzucker, 2002). The application of urea based fertilizer which upon hydrolysis confers NH_4^+ related characteristics has recently been gaining popularity (Miller and Cramer, 2004). However, mixed N nutrition occurring in the presence of both NH_4^+ and NO_3^- is capable of supporting better crop growth than sole application of either N form (Cramer and Lewis, 1993).

1.3 Limitations of nitrate based fertilizers

Nitrate availability in soil is greatly constrained by its high mobility rate which facilitates its rapid loss. Its loss mainly occurs through leaching and denitrification which reduces its availability within the rhizosphere. In nature, nitrification process whose end product is NO_3^- thus leads to appreciable N losses from agricultural land. Besides reducing nutrient availability for uptake, NO_3^- leaching potentially pollutes underground water bodies and also cause eutrophication of above ground water bodies through run-off. It is for these reasons that the current farming practices emphasize improved crop yields in a benign environment through a sustainable production system. The main purpose of improved farming method is to enhance food security to feed the ever increasing human population (Mangelsdorf, 1966; Vasil, 1998). This undertaking has opened up a window of research opportunity for the re-evaluation of previous agricultural practices. This is crucial since cereal production which provides the bulk of edible fibre consumes more than 60% of the total N fertilizers worldwide annually (Rao and Popham, 1999).

1.4 Agronomic requirements and economic importance of barley

Barley is a member of the grass family, Poaceae. It is a grain crop that is currently grown in more than 100 countries worldwide and is ranked fourth both in terms of quantity and area of production covered by cereals. It grows best on light soils rich in minerals with moderate water requirements. It is tolerant to cold stress and resistant to soil salinity than wheat. It exists either as winter or spring crop and its cultivars largely occur as six or two row grained ears. It serves as human food such as bread and other cereal products besides its usage as livestock fodder while green or hay when dry and silage in the conserved form. Dry straw can be used as animal bedding while grains can also be processed into animal feed concentrates. Several industrial brews such as beer, whiskey and malt syrup are among important alcoholic drinks manufactured from barley which are consumed by a large human population. The crop generates a lot of income to economies of countries such as USA, China, Canada, United Kingdom, Germany and Netherlands among others that produce and process its products (Foster, 1981).

1.5 Principle of CULTAN fertilization technique

The new fertilization method is based upon the injection of concentrated liquid NH_4^+ into soil and its subsequent adsorption onto clay particles and soil organic matter which enhance its long-term availability for crop uptake (Sommer, 1995). The fertilization method is developed within the tenets of emerging crop production paradigm, which emphasize improved crop yields in a sustainable manner. The adoption of such agricultural practice does not compromise ecological and environmental quality and it plausibly sustains and improves crop yields. When adsorbed onto clay soil matrix, NH_4^+ forms a sorption-complex which serves to regulate its uptake by inhibiting root growth and penetration into the concentrated zone (Zhang and Rengel, 2000). In Germany, the fertilization technique is very popular and is referred to as CULTAN. The acronym 'CULTAN' is an abbreviation of Controlled Uptake Long Term Ammonium Nutrition (Sommer, 1993; 2000; 2003). Similar methods are being practised elsewhere, though they are known by different names. For example, it is referred to as point injection in Canada and America (Janzen et al., 1991) while in Asia, more specifically Japan, sulphur-coated nitrogen are applied as slow release fertilizers and are commonly referred to as Controlled Nitrogen Release Fertilizers (CNRF) (Wakimoto, 2004). Adoption of the CULTAN fertilization method promotes localized NH_4^+ deposits within the rooting zone whose toxicity causes root inhibition into the injection-point whereas the less toxic peripheral zones promote intensive root growth network (Sommer, 2000).

1.6 Dilemma of inadequate and excess nitrogen nutrition

In most tropical soils, N is insufficient for satisfactory crop yield outputs. On one hand, the use of fertilizer inputs in developing countries, especially in sub-Saharan Africa, is quite low with essential nutrients often inadequately supplied due to high input costs, unavailability and poor marketing infrastructure (Dakora and Keya, 1997). On the other hand, developed countries are faced with environmental pollution resulting from excessive amounts of NO_3^- fertilizer applied in an effort to improve crop yields (Jeuffroy *et al*., 2002). Whereas developing countries are mainly faced with fertilizer inadequacy, developed countries are experiencing excessive fertilizer application problems with the potential of causing environmental pollution. A middle ground for both the developed and developing countries may be struck through improved fertilization techniques such as

CULTAN technique which has the ability of improving N availability for crop uptake during the cropping period. When fine-tuned, the fertilization technique may alleviate constraints associated with fertilizer input inadequacies of developing and excesses experienced in the developed worlds.

1.7 Potential of nitrification inhibitor incorporation into CULTAN

A potential N loss mitigation method that would be compatible with CULTAN fertilization technique is the incorporation of nitrification inhibitors into soil injected ammonium. Nitrification inhibitor (NI) incorporation into ammonium fertilizer can temporarily improve nitrogen retained as NH_4^+ through the suppression of the nitrification process (Crawford and Chalk, 1993). Application of NI influences not only fertilizer efficiency by reducing leaching or denitrification losses, but also the ratio of available inorganic N forms (Vanneli and Hooper, 1992). Occasionally, immobilization of N increases in response to NI-incorporation into soil thereby reducing N available for crop uptake (Vanneli and Hooper, 1993). However, the potential benefits accruing from NI-incorporation into NH_4^+ fertilizer through the suppression of nitrification supersedes the corollary effect of N losses when no nitrification inhibitor is incorporated. One of the commercially available nitrification inhibitors reported to successfully reduce nitrogen losses through less gas emissions is Nitrapyrin® (McCarty, 1999).

1.8 Merits of CULTAN fertilization technique

The popularity of CULTAN fertilization technique is due to the merits associated with its application. The method bases upon NH_4^+ as the dominant N form, which inhibits root penetration through the injection depot. This occurs because of its toxicity to roots. Proliferation of intensive root network around the injection zone is thought to trap diffusing ions, while the root growth inhibition by free ions within the injection-point offers a self-regulatory mechanism for N uptake as opposed to conventional nitrate fertilizer application that is made in splits to coincide with crop growth stages deemed by the farmer to require increased nutrient supply, which may, however, not be the case based upon the internal crop nutrient status (Sommer, 2000). Since NH_4^+ assimilation within the crop roots requires translocation of carbon skeletons from shoot to root, it offers a further regulatory measure because the uptake has to be determined by the proportion of C-skeleton translocation to the roots (Cramer and Lewis, 1993). The resulting organic nitrogenous compounds are thus incorporated into root system or channelled to other parts including the shoot. The supply of C-skeletons through photosynthesis and translocation of photosynthate to the root in conjunction with the redistribution of root assimilated nitrogenous compounds creates a counter-current trafficking of the growth essentials in opposite directions which result in a source-sink relationship for different products that are highly dependent upon each other. Along side these physiological merits, the advantages attained through the use of mechanized farm equipments which facilitate the wide adoption of CULTAN fertilization technique in large scale and in different crops are some of the factors that have contributed to its popularity.

1.9 Objectives of the study

The study focused on generating information to bridge the knowledge gap between crop and microbial responses in CULTAN-fertilized soils. In addition, crop growth and yield responses resulting from the interaction effects of CULTAN fertilization and different growth temperatures have not been evaluated to date. To be able to exploit the full potential of CULTAN-fertilization method, its interaction with abiotic and biotic factors need to be addressed. Currently, various mechanisms and processes relating to CULTAN-fertilization are not well understood and some are at best only assumed. Though it is known that NH_4^+ adsorbs onto clay particles and soil organic matter to form sorption-complex within the injection-point (Sommer, 2000), it is however, not known how stable the injected NH_4^+ is within the soil. In case the injected NH_4^+ is stable to any extent, it is unknown for how long it would support crop growth. Such information on NH_4^+ stability can help in decision making whether a single injection at a certain concentration would be sufficient to support crop growth throughout the entire season or whether there would be need for multiple injections to be performed at intervals coinciding with particular crop growth stages. Therefore, the first objective of this study was to evaluate the stability of soil-injected liquid ammonium and crop growth responses.

No data is available to support the view that highly concentrated NH_4^+ is toxic to soil microbes, especially bacteria. This presumption seems to stem from previous reports highlighting root growth inhibition by concentrated NH_4^+ fertilization (Sommer, 2000). Chemolithoautotrophic bacteria are known to use ammonia as an energy source during oxidation when they transform ammonia to nitrate via nitrite (Purkhold et al., 2000). There is however, no experimental data to illustrate bacterial community responses to soil-injected liquid NH_4^+. In light of this information gap, the second objective of the study focused on the assessment of the occurrence and response of bacterial communities associated with soil-injected liquid NH_4^+ analyzed by targeting 16S rRNA gene. Attempts to detect occurrence and abundance of ammonia oxidizing bacteria (AOB) while targeting ammonia monooxygenase subunit A (*amoA*), functional gene, were also made.

The direct influence of temperature on plant growth caused by effects on root mineral nutrient and water uptake as well as translocation is well known (Macduff and Jackson, 1991). In addition, the effect of temperature on microbial activity, particularly ammonia oxidation rates has been reported (Avrahami et al., 2003). However, no study has been conducted to elucidate the interaction of the two factors under a cropping system fertilized through the CULTAN method, which is predominated by NH_4^+ over NO_3^-. There is a high possibility of growth temperatures and N form available for absorption by crops interacting with each other, especially in the presence of mixed N nutrition resulting from the nitrification of soil-injected NH_4^+ to cause a suite of growth responses with strong implications on grain yield and yield forming factors. In this regard, the last objective of this study was to evaluate the impact of liquid NH_4^+ on barley yield and yield forming factors under different growth temperatures. In summary, the following specific objectives were addressed by the study:-

i) To evaluate stability of CULTAN-injected NH_4^+ and its effect on barley crop growth.
ii) To assess occurrence and characterize bacterial community responses to CULTAN fertilized soils.
iii) To investigate occurrence and abundance of ammonia oxidizing bacteria (AOB) in CULTAN-fertilized soils.
iv) To elucidate the interaction effects of soil-injected NH_4^+ and growth temperatures on barley grain yield and yield-forming factors.