ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF
AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Agronomy 1(1): 54-59, 2002 **January-March 2002**

Added Nitrogen Interaction in the Soil-Plant System–A Review

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Abstract: Application of fertilizer N to soil or to the soil-plant system often leads to enhanced mineralization and plant availability of N. By using ^{15}N isotope methodology, it has been found that the extra N comes from soil organic matter as a result of interaction of the added N. This phenomenon is termed "priming" action or added nitrogen interaction (ANI) and may be apparent or real and positive or negative. Apparent ANI is supposedly caused by pool substitution, while real ANI results from changes in the processes that move N into or out of a given pool. Although ANI is generally positive, negative ANIs may arise from processes like net immobilization, denitrification and NO_3^- leaching. Occurrence of ANI has implications to the determination of fertilizer use efficiency as well as to the fate of fertilizer and soil N. Hence, an understanding of the occurrence of ANI and the mechanisms involved is necessary to devise strategies for improved fertilizer management practices.

Key words: Nitrogen, ^{15}N, N immobilization, N mineralization, N uptake, priming effect

Introduction

Nitrogen is the key nutrient element limiting crop production under most situations. Use of chemical N fertilizers has therefore resulted in substantial increase in crop production over the past few decades. Presently, world agriculture uses 42 million tones of fertilizer nitrogen a greater part of which is consumed by crops like wheat, rice, sugarcane and cotton. In most situations, however, efficiency of fertilizer N use by plants is fairly low ranging from 30 to 50% of the applied, while 20-70% is lost from the soil plant system (Hauck, 1985). This low use efficiency is not only of concern from economic reasons, but different forms of nitrogen that find their way into the environment (soil and atmosphere) have serious negative implications (Crutzen, 1981; Bouwman, 1990). Fortunately, however, low use efficiency of fertilizer N is compensated to a certain extent by an increase in N availability from sources like soil organic matter and root-mediated biological nitrogen fixation. This increase is attributed to a priming action or priming effect of the added nitrogen. Alternatively, the added nitrogen interacts with the native soil N in a way to increase the availability of the later.

Lohnis (1926) was the first to observe and report the stimulating effect of added materials on the turnover of native organic matter. He observed increased mineralization of native organic N in soil following the addition of green manures. Subsequent experiments by Broadbent and co-workers (Broadbent and Bartholomew, 1948; Broadbent and Norman, 1946) clearly demonstrated an increase in the mineralization of soil C and N following addition of plant residues and mineral N. However, the effect may not always be positive since a decrease in mineralization has also been reported (Nicholardot *et al.*, 1986). The term "priming effect" was introduced by Bingemann *et al.* (1953) to interpret such phenomena, which may therefore be both positive and negative. In their review, Jenkinson *et al.* (1985) introduced the term "added nitrogen interaction" or ANI to describe any effect that the addition of N may have on the N already present in the soil. This terminology has been extensively used in subsequent studies. Recently, however, Kuzyakov *et al.* (2000b) have opted to use "priming effects" to interpret "strong, short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil". These treatments may include input of organic or mineral fertilizer to the soil, exudation of organic substances by roots, mechanical treatment of soil, or drying and wetting cycles etc.

The occurrence of ANI and the mechanisms involved have been of considerable research interest in view of its impact on N economy of agro ecosystems and N nutrition of plants. Consideration of ANI is essential also because of its possible effects on N loss to the environment, particularly the leaching of NO_3^- and denitrification (Baraclough *et al.*, 1984; Wu *et al.*, 1991). Use of ^{15}N methodology has greatly helped in understanding such interactions and their implications to interpretation of experiments using ^{15}N-labelled fertilizers (Jenkinson *et al.*, 1985). The use of ^{15}N-labelled fertilizers is particularly important when the objective is to study the plant uptake (or mineralization in soil) of fertilizer N or non-fertilizer N. In such studies, it is because of the ANI that higher values of fertilizer use efficiency are generally obtained by difference method as compared to ^{15}N isotopic method (Jansson, 1958; Torbert *et al.*, 1992; Azam *et al.*, 1993c). This paper reviews the literature on positive added nitrogen interaction (ANI) with special reference to studies reported from Pakistan.

Occurrence of added nitrogen interaction

Changes in the extractability of soil N in response to application of carbon and nitrogen: Extractability of soil N has often been used as an index of soil N availability (Jenkinson, 1968; Fox and Peikilek, 1978; Stanford, 1982; Sahrawat, 1982). In general, the use of mild extractants including 10mM $CaCl_2$ and $NaHCO_3$ has been favored (McGill and Paul, 1976; Keeney, 1982; Stanford, 1982). Michrina *et al.* (1982) characterized the organic matter extracted from soil with 10mM $NaHCO_3$ and hot 10mM $CaCl_2$. They concluded that each extractant provided an index of available N by removing a specific but poorly defined fraction of soil organic matter. Stanford (1982) suggested that N removed by mild extraction procedures and the N mineralized during incubation are derived from a common source. Using ^{15}N methodology, Juma and Paul (1984) found a close relationship between biomass N and the amount of ^{15}N-labelled organic N mineralized or that recovered as NH_4-N by mild extraction. Since mineralizable N may be proportional to the size of the biomass (Jenkinson *et al.*, 1968), the best extraction procedure will be that which selectively extracts biomass N. Kelley and Stevenson (1985) produced ^{15}N-labelled microbial biomass in soil and found acidified permanganate and anhydrous formic acid to be the best extractants of biomass N. A close relationship is indeed reported between N contained in microbial biomass and that actually taken up by the plants (Jenkinson and Ladd, 1981). Hence, biomass N determined by extraction procedures can serve as a good measure of plant available N.

In spite of the significance of chemical extracted N in determining the plant available N, very few, if any, studies have been reported on the effect of amendments on extractability of soil N and thus its subsequent availability to crop plants. Azam *et al.* (1989b) were probably the first to incubate a soil at different levels of added nitrogen and studied the extractability of native soil N as affected by oven-drying, freeze-drying and fumigation. In their study a silt-loam soil from Pakistan was incubated with increasing amounts (N applied at 67, 133, 200, 267 and 333μg g^{-1} soil) of ^{15}N-labelled ammonium sulphate and glucose (C to N ratio of 30 for all additions). Rate of immobilization of applied N and onset of remineralization was followed for 460 hours. At a stage when all the applied ^{15}N was in microbial biomass and products, soil samples were refluxed with 10mM $CaCl_2$, extracted with 10mM $NaHCO_3$, extracted with 500 mM K_2SO_4 after chloroform fumigation, freeze-drying and oven-drying.

Of the three extractants used i.e., $CaCl_2$, $NaHCO_3$, and K_2SO_4, the former extracted maximum percentage of the soil N which ranged from 4.5 to 7.2% at different levels of applied N; 2.4 to 5% of the soil N was extracted with other two extractants. Extractability of soil N increased with the amount of N applied, maximum increase being observed at the highest rate of N addition i.e., 333μg g^{-1} soil. The increase in N extracted with $NaHCO_3$ and K_2SO_4 was consistent with the amount of applied N. In the case of $CaCl_2$, rates of N addition higher than 67μg g^{-1} soil caused a significant but statistically similar increase in extractable N. It appeared that $CaCl_2$ had already extracted maximum soil N at an addition rate of 133μg g^{-1} soil. In addition, $CaCl_2$ may not be very selective for the labile N component of soil organic matter i.e., microbial biomass. Of the three extractants, K_2SO_4 was the least effective and could thus be considered as relatively mild and more selective for extracting labile N. Kelley and Stevenson (1985) also found $CaCl_2$ to be less effective in extracting biomass N. Nevertheless, the amount of N extracted with all three extractants increased with the rate of applied N suggesting a priming effect or ANI.

Soil treatments like oven-drying, freeze-drying, and chloroform fumigation caused a substantial increase in the extractability of soil N. Maximum increase in the extractability of soil N was caused by chloroform fumigation and up to 6.5% of the soil N was extracted. Soil samples subjected to physical and chemical treatment are often reported to show an increased in the mineralization and extractability of soil N (Jenkinson, 1966; Shields *et al.*, 1973; Powlson and Jenkinson, 1976; Marumoto *et al.*, 1977). In the study reported by Azam *et al.* (1989b), however, extractability of soil N increased with the amount of applied N, again suggesting a positive ANI. In another study, Azam *et al.* (1989a) reported 2.78% of the native soil N extracted at 67 μg N g^{-1} soil and 3.35% at an addition rate of 333 μg N g^{-1} soil. This increase could be attributed to an increase in microbial proliferation at the expense of applied C and N and transformation of a part of native soil N into microbial biomass and thus rendered more extractable than the non-biomass N. Since, microbial biomass increased with the amount of applied C and N, a consistently higher amount of native soil N would have been transformed into microbial biomass and hence extractable. It would appear therefore that by increasing the microbial biomass and activity, the amendments would mobilize the otherwise recalcitrant native soil N, the effect being more at higher rates of application.

Mineralization of native soil N in response to application of fertilizer N and plant residues: As mentioned earlier, mineralization of soil N is increased following application of fertilizer N (Broadbent, 1965; Legg and Stanford, 1967; Hauck and Bremner, 1976; Jenkinson *et al.*, 1985; Hart *et al.*, 1986; Woods *et al.*, 1987; Chalk *et al.*, 1990; Clay and Clapp, 1990). Azam and co-workers have pioneered such studies in Pakistan. In an experiment conducted under laboratory conditions, Azam *et al.* (1989b) reported a significant increase in the mineralization of native soil N (2.56 and 4.71% of soil mineralized at 67 and 333 μg N g^{-1} soil, respectively). Under anaerobic conditions as well, mineralization of native soil N was consistently more at higher rates of N addition i.e., 2.90 and 4.44% of the soil N at N addition rates of 67 and 333 μg N g^{-1} soil, respectively. There was a close correlation between N mineralized from soil organic matter under aerobic and anaerobic conditions ($r = 0.87$). Ammonium applied to two soils from Pakistan also caused a significant ANI that increased with the amount of applied N (Azam *et al.*, 1993a). In another study (Azam *et al.*, 1993b), three Illinois Mollisols were incubated for 2 weeks at 25 °C after treatment with different amounts of glucose and/or ^{15}N-labelled $(NH_4)_2SO_4$ or ^{15}N-labelled KNO_3. The objectives were I) to compare the immobilization and interactions of NH_4^+ and NO_3^- with the native soil N and ii) to study the relationship between immobilization of applied N and the ANI. In all cases, both NH_4^+ and NO_3^- were actively immobilized and transformed into organic forms in the presence of glucose. Although the three soils differed in the extent of applied N immobilized, trends were similar. A positive ANI was observed in all soils, the magnitude increasing with the rate of applied N as also reported by other (Broadbent and Nakashima, 1971). In the absence of glucose, a higher ANI was observed for NH_4^+ than NO_3^-, an observation in line with several other studies (Jansson, 1958; Rennie and Rennie, 1973; Kowalenko and Cameron, 1978; Steele *et al.*, 1980; Hart *et al.*, 1986; Stout, 1995).

In the presence of glucose, however, the differences were not significant. Significant correlations were observed between applied N immobilized and the ANI only in one soil having a high native mineral N content. Generally, the amount of applied N immobilized was lower than the ANI suggesting the later to be real at least partly.

Studies by Azam *et al.* (1991a) suggested that apparent ANI may not be observed in soils devoid of mineral N (e.g., due to plant growth or chemical extraction). This suggestion was confirmed in subsequent studies in which native mineral N was removed (using mild extraction with 0.01M $CaCl_2$) from or retained in 6 soils (1994a). From 6 to 49% of the N applied as ^{15}N-labelled $(NH_4)_2SO_4$ was immobilized during 14 days of incubation; immobilization being higher in extracted soil. A positive ANI was observed that exceeded the amount of applied N immobilized in unextracted soil and increased with the addition of N. In extracted soil, however, immobilization of applied N was more than that in unextracted soil, reverse was true for ANI. Thus a higher immobilization of applied N may not necessarily be accompanied with a higher ANI, a requirement for the ANI to be apparent.

Like mineral fertilizers, plant residues with high N content and a narrow C/N ratio are also reported to cause a net increase in the mineralization of native soil N. In fact, the first reports on this phenomenon were from studies using leguminous plant residues (Lohnis, 1926; Broadbent and Bartholomew, 1948; Broadbent and Norman, 1946). In a laboratory incubation experiment, plant residues of soybean, corn, and vetch were found to enhance the mineralization of native soil N (Azam *et al.*, 1993c). In this study, both negative and positive ANI was observed. Negative ANI was attributed to I) an initial immobilization of native soil N and its subsequent stabilization into recalcitrant organic compounds and ii) denitrification in the presence of easily oxidizable C applied as plant residues. It was suggested for the first time that one of the benefits of applying N rich green manures may be the enhanced availability of soil N through ANI. In an earlier study using rice and wheat as indicator crops, enhanced mineralization of native soil N or ANI was considered as one of the major factors in enhancing crop yields following amendment of soil with leguminous green manures (Azam, 1990).

Added nitrogen interaction and availability of soil N to plants: As discussed above, ANI leads to an increase in the extractability and mineralization of native soil N. This increase is also reflected in plant experiments using isotopic as well as non-isotopic techniques for the determination of fertilizer use efficiency. However, when using non-isotopic techniques, differentiation between sources of N cannot be made and exaggerated values of fertilizer use efficiency are obtained compared to those obtained with isotopic method (Jansson, 1958; Torbert *et al.*, 1992; Azam *et al.*, 1993c). The increase in the uptake of unlabeled N (when applied N as ^{15}N-labeled) or labeled N (when soil N is ^{15}N-labeled) is generally believed to arise from immobilization-driven pool substitution (apparent ANI) or form increased soil volume being explored by the roots (real ANI). Mechanisms responsible for ANI are discussed later.

In order to differentiate between sources of plant N, use of ^{15}N-labelled fertilizers has proved to be a convenient tool. Such a differentiation is essential to devise strategies for economizing N as well as preserving the environment from hazardous side effects of excessive applications of fertilizers. These effects include I) contamination of ground water with NO_3^-, ii) eutrophication, and iii) emissions to atmosphere of nitrogen oxides with implications to global temperatures and stability of ozone layer etc.

When using ^{15}N-labelled fertilizers for determining their fate in the soil-plant system, an increase in the uptake of unlabeled N by plants through the so-called "priming" effect or ANI has often been reported (Jenkinson *et al.*, 1985, Hamid and Ahmad, 1993; Kuzyakov, 2000a). There is experimental evidence that ANIs increase with the rate of applied N (Legg and Stanford, 1967; Hart *et al.*, 1986). However, ANIs may not always be positive. Campbell and Paul (1978) and Bigeriego *et al.* (1979) have reported negative ANIs which are interpreted to arise from I) excessive use of fertilizers (Jenkinson *et al.*, 1985) or ii) replacement of nutrient losses from soil organic matter (Kuzyakov *et al.*, 2000b).

In a comparative study using ^{15}N-labelled organic and inorganic N sources for rice, Azam (1990) reported a substantial ANI and suggested this to one of the mechanisms whereby leguminous plant residues enhance plant growth. However, the crop varieties may vary in supporting ANI, the variation being attributable to root proliferation (Azam *et al.*, 1991b; Azam, 1992). Similarly, the extent of ANI may differ depending upon the stage of plant growth at which fertilizer N is applied. Ashraf and Azam (1998) reported significant differences in wheat varieties in exhibiting ANI that also differed due to time of N application. In another study, a significant correlation ($r = 0.89$) was observed between soil N uptake and dry matter yield of wheat (Azam *et al.*, 1990) suggesting that factors affecting availability of soil will have an important bearing on crop production. Thus amendment of soil with plant residues with a wide C/N ratio will retard plant growth mainly by reducing the availability of soil N rather than fertilizer N (Azam *et al.*, 1993d). Similarly, the soil factors that affect the mineralization and thus the plant availability of soil N will lead to a reduction in crop production. In a greenhouse experiment, Azam *et al.* (1992) studied the effect of different salt and fertilizer N levels on the growth of flooded rice. Uptake of soil N was highly inhibited due to salinity, while fertilizer N exhibited the phenomenon of ANI; the values of ANI being greater at higher levels of added N. The ANI was found to be real as it was greater than the fertilizer N immobilized and the root biomass was more in fertilized plants; contribution of apparent ANI was found to be fairly small. A part of ANI was suggested to arise from rhizospheric N_2 fixation conditions which were fairly conducive under flooded conditions.

As mentioned earlier, NH_4^+-N causes higher ANIs compared to NO_3^-. Thus use of nitrification inhibitors may prolong the availability of NH_4^+-N in soil leading to higher ANIs. In a greenhouse study, Lodhi *et al.* (1996a,b) reported the occurrence of ANI following application of a nitrification inhibiting insecticide to rice and maize. The ANI was attributed to I) prolonged availability of NH_4^+-N, ii) enhanced mineralization of soil N and iii) greater root biomass.

The mechanisms responsible for added nitrogen interaction: Jenkinson *et al.* (1985) have described in detail the theoretical aspects of ANI. This effect of physical treatments or fumigation etc. has been attributed to I) exposure of otherwise inaccessible organic matter to microbial attack (Rovira and Greacen, 1957), ii) release of cellular components (Marumoto *et al.*, 1977), iii) partial or complete sterilization followed by mineralization of dead microbial cells by surviving or newly inoculated microorganisms (Jenkinson, 1966). It is likely that all three mechanisms function simultaneously.

ANIs can be real or apparent and either positive or negative and could occur simultaneously in the same pool. Real ANI is that in which fertilizer N causes a change in the processes that move N into or out of a particular compartment. It can be positive if there is an increase in the soil N in a compartment and negative if the result is otherwise. Likewise, ANI will be real and positive if fertilizer N leads to an increase in the volume of soil being explored by roots and hence an increase in the uptake by plants of native soil N. ANI will be apparent if caused by pool substitution or isotope displacement reactions. Pool substitution is the process by which added N stands proxy for native unlabeled N that would otherwise have been removed from that pool. Microbial immobilization of N, whether driven by the decomposition of soil organic matter or by the decomposition of plant roots, can lead to pool substitution and is the dominant cause of the apparent ANIs. Denitrification and plant uptake of N can also, under special circumstances, lead to pool substitution and thus give rise to apparent ANIs. Isotope displacement reactions, in which the added labeled N displaces native unlabeled N from a bound pool can also lead to apparent ANIs but are only likely to be of significance in exceptional circumstances. A positive apparent ANI is accompanied by an "A" value that changes as fertilizer applications increase. Likewise, a positive "apparent" ANI also causes fertilizer uptake efficiency to appear lower when measured by the uptake of ^{15}N than when measured with the non-isotopic difference method. Several workers have reported higher fertilizer use efficiency determined by difference method as compared to isotopic method (Jansson, 1958; Torbert *et al.*, 1992; Azam *et al.*, 1993c).

According to Jenkinson *et al.* (1985), apparent ANI may result from displacement reactions. This concept may be valid so far as the difference in NH_4^+ and NO_3^- in causing an ANI is concerned since the former is reported to cause a higher ANI than the later. This means that added NH_4^+ gets exchanged with the native recalcitrant or bound NH_4^+. Logically, such reactions would lead to a simultaneous incorporation of added and release of native N into the mineral or plant available pool. When using non-isotopic methods, this extra N mineralized in soil or taken up by plants will mistaken as originating from the added nitrogen. Because of the recalcitrance of relatively stabilized soil N (bound N), NH_4^+ on the exchange complex or relatively labile biomass N will be more susceptible to such an exchange or displacement. However, the spurious increase in the release of soil N or its uptake by plants during a short course of time can hardly be explained on the basis of displacement reactions. Broadbent and Nakashima (1971) took exception to the concept that sufficient unlabeled fixed NH_4^+ could be displaced from the soil NH_4^+ to produce a measurable apparent ANI. In a laboratory experiment using 3 Illinois Mollisols, Azam *et al.* (1994a) observed a very little exchange between applied $^{15}NH_4^+$ and the native clay-fixed NH_4^+. They suggested that variations in NH_4^+ fixation capacity of soils will not have a significant bearing on the interpretation of data obtained from studies of the ANI.

Another possibility is that applied NH_4^+ gets exchanged with that in the microbial biomass. Normally, however, upon entry into the microbial cells, NH_4^+ is incorporated almost instantly into the amide group of glutamine and into the major biochemical pathways. Hence exocellular NH_4^+ may exchange with amide group, but the resultant ANI cannot be appreciable in quantitative terms especially because microbial biomass contains only a small proportion of the soil N. Hence mechanisms other than displacement reactions may be involved. Most probably through immobilization-remineralization reactions the applied NH_4^+ gets incorporated into the microbial biomass along with the native soil N followed by the release of the later into the inorganic N pool in soil (Steele *et al.*, 1980) giving the impression of ANI.

Immobilization-driven pool substitution could be an important mechanism responsible for an apparent ANI. Jenkinson *et al.* (1985) defined pool substitution as the process by which labeled N (^{15}N) added to a particular pool takes the place of unlabeled (^{14}N) soil N (or in other words stands proxy for the unlabeled soil N) that would otherwise have been abstracted from that pool. In order for the pool substitution to occur it is essential that I) the conditions in soil are conducive for a net immobilization, and ii) microbes discriminate ^{15}N (added) against ^{14}N (native). The first condition is easily met in soils containing sufficient quantities of easily decomposable organic matter or following addition of fresh plant residues. Laboratory incubations have shown that considerable quantities of applied N are immobilized even if there is a net mineralization of N (Broadbent and Nakashima, 1971; Shen *et al.*, 1984). However, the second condition is hardly met if we accept the suggestion of Hauck and Bremner (1976) that no significant discrimination for ^{15}N and ^{14}N is made by the microorganisms during N assimilation. Contrary to such a suggestion, it is quite well known now that isotopic discrimination certainly occurs both in chemical and biochemical reactions. The underlying principle for producing ^{15}N-labelled material rests on the premise that if oxides of nitrogen are passed through nitric acid, the emerging gas will be depleted while the nitric acid will get enriched. It is also known that soil nitrogen is relatively high in ^{15}N content due mainly to the preferential loss of nitrogen by processes like denitrification. It is this difference in ^{15}N content of soil N and atmospheric N that serves as the basis for estimating biological nitrogen fixation using isotopic dilution method. The occurrence of isotopic discrimination during immobilization will thus result in negative instead of positive apparent ANI.

If immobilization-driven pool substitution is accepted as the mechanism for the observed ANIs, then the fertilizer N immobilized should be equal to the ANI. This is, however, not the case in most of the reported studies that show substantially higher ANI than the amount of applied N immobilized (Azam *et al.*, 1994b). Further, addition of NH_4^+-N to a soil deprived of mineral N should not exhibit an ANI as there is nothing to stand proxy for. Results contrary to this have, however, been reported and a substantial

ANI has been reported for soils exhausted of their native mineral N content (Azam *et al.*, 1994a). Another widely accepted concept originally proposed by Jansson (1958) is that NH_4^+ is assimilated by the soil microorganisms in preference to NO_3^-. Legg and Stanford (1967) and Craswell and Strong (1976) observed very little immobilization of NO_3^- -N in fallow soils. Hence addition of NO_3^- to a soil immobilizing N is not expected to cause an ANI. Some other studies, however, not only show a net immobilization of NO_3^- -N, but a positive ANI as well (Azam *et al.*, 1988, 1993b). Zagal and Persson (1994) also observed rapid immobilization of NO_3^- and a positive ANI in the presence of glucose. It follows therefore that immobilization-driven pool substitution cannot be the only process involved (Westerman and Kurtz, 1973). Nevertheless, the occurrence of pool substitution is supported by the reported increases in the size of the ANI with the amount of applied N (Broadbent and Nakashima, 1971; Azam *et al.*, 1991a). Simple mathematics will show that with increase in the quantity of applied N there will be a decrease in the amount of native N immobilized leaving progressively higher amounts to be detected in soil mineral N pool and hence a positive ANI. Again, the amounts will still be high enough to suggest pool substitution as the dominant mechanism responsible.

From the above it would appear that the ANIs termed apparent cannot realistically be attributed to pool substitution through displacement reactions and immobilization. Alternatively, however, denitrification has been suggested as leading to apparent ANI (Jenkinson *et al.*, 1985) following addition of labeled NO_3^-. However, as mentioned earlier, ANI is generally more obvious following addition of NH_4^+ rather than NO_3^-, while addition of the former is reported to cause a higher denitrification of any NO_3^- added or that already present in the soil (Azam *et al.*, 2001). This increase has been attributed to the development of anaerobic microsites especially in the presence of NH_4^+ that encourages microbial proliferation more than NO_3^-. Under these circumstances, ANI will be negative rather than a positive apparent ANI. Nevertheless, a positive apparent ANI in NO_3^- - treated soil in the presence of glucose has been reported and attributed to immobilization-driven pool substitution (Zagal and Persson, 1994). In acid soils, mineralization of N can be accelerated by raising the pH e.g., by applying urea. In addition, high salt concentrations kill the soil microorganisms thereby releasing additional mineral N. Broadbent and Nakashima (1971) found NH_4^+ salts to be more effective in accelerating the mineralization of N. They ascribed this increase to pH effects. Jenkinson *et al.* (1985) persist with the idea that large ANIs observed in case of NH_4^+ are largely apparent although no really sound evidence is available to support this contention. Studies do show, however, that mineralization of organic N is enhanced by added fertilizer N especially NH_4^+ (Broadbent and Nakashima, 1971; Azam, 1990), negating the concept of apparent ANI.

In view of above considerations, the mere occurrence of an apparent ANI proposed by Jenkinson *et al.* (1985) becomes questionable. Hence, any increase in the extractability, mineralization, and plant availability of unlabeled N from soil following application of labeled N (or vice versa) should be considered real that results from the change in the processes that move N into or out of a particular compartment as aptly defined by Jenkinson *et al.* (1985). However, according to these authors only in exceptional cases does the addition of inorganic N to a soil accelerates the mineralization of soil organic N and the associated release of CO_2 and consumption of O_2. Usually the net mineralization is either the same or lower in N amended soil than in the control (Jansson, 1958; Shen *et al.*, 1984). Other research has shown that N fertilizer additions consistently reduced rather than stimulated CO_2 production in field soils (De Jong *et al.*, 1974). Kowalenko *et al.* (1978) also showed that incubated samples of N fertilized soil consumed less O_2 than did samples of control soils. These results would suggest that microbial activity is retarded in fertilized soils. This is not, however, the case. Under fertilized conditions, a higher amount of C is transformed into microbial biomass and products rather than respired i.e., efficiency of C use and transformation is increased (Zagal and Persson, 1994). Under N deficient conditions (unfertilized soil), turnover of C is much more rapid leading to a higher loss of C. Therefore a decrease in C mineralization cannot be ascribed to a negative effect of fertilizer. Rather a positive effect in terms of microbial proliferation and metabolism may be more probable thereby leading to a real positive ANI. Thus increased microbial activity following addition of fertilizer N (especially NH_4^+-N) could be considered as the main mechanism responsible for ANI which is real under most circumstances.

Controversy regarding the nature and mechanisms of ANI in experiments involving plants is similar to that discussed for non-plant studies. There is no doubt, however, that uptake by plants of unlabeled N is increased following application of ^{15}N-labelled fertilizers. This enhancement is evident from the fact that use efficiency of fertilizer N determined by non-isotopic difference method is generally higher as compared to that with isotopic method (Jansson, 1958; Torbert *et al.*, 1982; Azam *et al.*, 1993c). This difference is certainly attributable to ANI that may be apparent or real. Again, apparent ANI is ascribed to immobilization-driven pool substitution (Jenkinson *et al.*, 1985) with plant roots providing necessary substrates in the form of rhizodeposits for N immobilization to occur. Arguments similar to those stated above hold true in this case as well and the ANIs observed in plant experiments will be termed real.

Stimulation in root proliferation has been suggested as the mechanism responsible for this real ANI (Hills *et al.*, 1978). A clear evidence for this was provided in a split-root experiment by Sapozhnikov *et al.*, 1968). Enhancement in root proliferation due to applied N has been reported (Sorenson, 1982). Some other studies, however, show that root biomass may not necessarily be responsible for enhanced N uptake as pruned and restricted roots may be equally effective (Andrews and Newman, 1970; Burns, 1980). Nevertheless, roots do exert a positive effect on the mineralization of soil N through increased microbial activities at the expense of rhizodeposits (Haider *et al.*, 1987; Zagal, 1994; Kuzyakov, 2000a). According to Kuzyakov (2000b) maximum priming effect appears approximately at the same time as the maximum of the activity or amount of microorganisms. Studies by Azam *et al.* (1989b, 1993c) clearly demonstrated an increase in the extractability and mineralization of native soil following addition of C and N. Root-induced N mineralization has also been reported that may increase with the increase in root proliferation. In addition, a healthier root system will support higher level of biological nitrogen fixation leading to increased uptake of unlabeled N and hence a positive real ANI. Contribution of root-supported biological nitrogen fixation and its contribution to ANI has not yet been explored.

Implications of ANI to fate of N in the soil-plant system: Added nitrogen interaction is the manifestation of using isotopic methods in soil-plant studies aimed at determining uptake/use efficiency of fertilizer N. This phenomenon leads to overestimates of fertilizer N use by plants when non-isotopic or difference method is employed. Thus determining fertilizer use efficiency simply by difference method will give a false impression of higher amounts of fertilizer N being used. Added N interaction leads to an increased mobilization of soil N reserves, while at the same time fertilizer N may be lost from the soil plant system. Indeed losses through denitrification and NO_3^- leaching may become an important manifestation of ANI (Kuzyakov *et al.*, 2000b; Baraclough *et al.*, 1984; Wu *et al.*, 1991). Added N, particularly NH_4^+ may also enhance the loss of NO_3^- through denitrification especially in the presence of easily oxidizable C (Azam, 2001). Such losses cannot be accounted for by the difference method. It is important to note, however, that ANI leads to mobilization of soil N resources which could be used to the benefit of the crop plants.

Application of fertilizer N leads to an increase in the mineralization and plant availability of N from relatively stable organic N component of the soil through ANI. In most cases using fertilizer N, the ANI will be positive. In exceptional cases of high C availability, removal of native soil NO_3^--N through immobilization and denitrification may lead to a negative ANI. Application of NH_4^+ or NH_4^+-forming fertilizers will lead to a higher ANI than NO_3^-. In most cases, the ANI is real and caused by I) increased microbial activities, especially the process of mineralization-immobilization turnover and ii) increased root activity enabling the plants to explore a greater soil volume for nutrient acquisition. Enhancing the process of ANI through organic amendment by exogenous application or through increased rhizodeposition will lead to increased availability of N to plants. However, an increase in N losses due to ANI is also possible. Use of isotopic methods seems essential not only to determine the fate of applied fertilizer N but also to devise improved fertilizer N management strategies and to reduce the loss of N.

Acknowledgments

This review was prepared during the research stay at the Institute of Applied Microbiology, Justus-Liebig University Giessen, Germany. The financial support provided by the Alexander von Humboldt Foundation, Germany, is gratefully acknowledged.

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