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 15N 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 NO3− 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, 15N, 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, 1988). 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 (Cruzen, 1981; Bouvyman, 1989). 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 effect 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 (1986) 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, 1948) clearly demonstrated an increase in the mineralization of soil C and N following addition of plant residues and mineral nitrogen. However, the effect may not always be positive since a decrease in mineralization has also been reported (Nicholardot et al., 1989). The term “priming effect” was introduced by Bingemann et al. (1983) 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, Kuzmakov 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 NO3− and denitrification (Barclough et al., 1984; Wu et al., 1991). Use of 15N methodology has greatly helped in understanding such interactions and their implications to interpretation of experiments using 15N labelled fertilizers (Jenkinson et al., 1985). The use of 15N 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 15N isotopic method (Jansson, 1978; 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 Peiklik, 1978; Stanford, 1982; Sahrawat, 1982). In general, the use of mild extractants including 10mM CaCl2 and NaOH has been favored (McGill and Paul, 1978; Keeney, 1982; Stanford, 1982). Michrina et al. (1982) characterized the organic matter extracted from soil with 10mM NaHCO3 and hot 10mM CaCl2. 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 15N methodology, Juma and Paul (1984) found a close relationship between biomass N and the amount of 15N labelled organic N mineralized or that recovered as NH4−N by mild extraction. Since mineralizable N may be proportional to the size of the biomass (Jenkinson et al., 1988), the best extraction procedure will be that which selectively extracts biomass N. Kelley and Stevenson (1985) produced 15N labelled microbial biomass in soil and found acidified permanganate and anhydrous formsic acid to be the best extractants of biomass N. A close relationship is also 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. (1999b) 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 slight-soil from Pakistan was incubated with increasing amounts of N applied at 67, 133, 200, 267 and 333mg g−1 soil of 15N 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 480 hours. At a stage when all the applied 15N was in microbial biomass and products, soil samples were refluxed with 10mM CaCl2, extracted with 10mM NaHCO3, extracted with 500 mM K2SO4 after chloroform fumigation, freeze-drying and oven-drying. Of the three extractants used i.e., CaCl2, NaHCO3, and K2SO4, the former extract 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
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soil N increased with the amount of N applied, maximum increase being observed at the highest rate of N addition i.e., 333 g g⁻¹ soil. The increase in N extracted with NaHCO₃ and K₂SO₄ was consistent with the amount of applied N. In the case of soil N addition higher than 67 g g⁻¹ soil caused a significant but statistically similar increase in extractable N. It was suggested that CaCl₂ had already extracted maximum soil N at an addition rate of 193 g g⁻¹ soil. In addition, CaCl₂ may not be very selective for the labile N component of soil organic matter (e.g., microbial biomass). Of the three extractants, K₂SO₄ was the least effective and could thus be considered as relatively mild and more selective for extracting labile N. Keeley and Stephenson, 1986 also found CaCl₂ to be more effective in extracting biomass N. Nevertheless, the amount of N extracted with all three extractants increased with the rate of applied N suggested 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 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 increase in the mineralization and extractability of soil N. (Wienker, 1986; Shields et al., 1973; Pavlov and Jenkinson, 1976; Marumoto et al., 1977). In the study reported by Azam et al. (1989), however, extractability of soil N increased with the amount of applied N, again suggesting a positive ANI. In another study, Azam et al. (1991a) reported about 2.78% of the native soil N extracted at 67 g g⁻¹ soil and 3.36% at an addition rate of 333 g g⁻¹ soil. This increase could be attributed to an increase in microbial population 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 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, 1986; Lawrence et al., 1986; Hauck and Ahuja, 1987; Jaepma et al., 1986; 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. (1989) reported a significant increase in the mineralization of native soil N as a result of N mineralization at 67 and 333 g 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 g g⁻¹ soil, respectively. Ammonium applied to two soils from Pakistan also caused a significant ANI that increased with the amount of applied N (Azam et al., 1989a). 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 ¹⁵N-labelled (NH₄)₂SO₄ or ¹⁵N-labelled KNO₃. The objectives were to compare the immobilization and interactions of NH₄⁺ and NO₃⁻ with the native soil N and II) to study the relationship between immobilization of applied N and the ANI. In all cases, both NH₄+ and NO₃- 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 amount of applied N. The amount of applied N was 500 mg N kg⁻¹ soil and Nakashima, 1971). In the absence of glucose, a higher ANI was observed for NH₄+ than NO₃-, an observation in line with several other studies (Janssen, 1988; 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 a higher level of 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 by mild extraction with 0.01 M CaCl₂ or retained in 6 soils (1994a). From 6 to 9% of the N applied as ¹⁵N-labelled (NH₄)₂SO₄ 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 extracted 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 ii) subsequent stabilization into recalcitrant organic compounds and deamination in the presence of easily oxidizable C applied as plant residues. It was suggested for the first time that one of the mechanisms of applying N rich plant residues 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 biomass as well as nitrification potential (Jaepma et al., 1986; Janssen, 1988; Trewartha and Hare, 1987). Ammonium is used 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 methods (Janssen, 1988; Trewartha and Hare, 1987; Azam et al., 1993). The increase in the uptake of unlabeled N (when applied N as ¹⁵N-labeled) or labeled N (when soil N is ¹⁵N-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 ¹⁵N-labeled 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 NO₃-, ii) eutrophication, and ii) emissions to atmosphere of nitrogen oxides with implications to global temperatures and stability of ozone layer etc. When using ¹⁵N-labeled 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, 1987; Hart et al., 1986). However, ANI may not always be positive. Campbell and Paul (1978) and Bgiergo 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 ¹⁵N-labeled 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 exudates (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 = 0.68) was observed between soil N uptake and dry matter yield of wheat (Azam et al., 1990) suggesting that factors affecting availability of soil N 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 markedly and reduce the availability of soil N rather than increase it (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 soil 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 N2 fixation conditions which were fairly conducive under flooded conditions. As mentioned earlier, NH4+-N causes higher ANIs compared to NO3--N. Thus use of nitrification inhibitors may prolong the availability of NH4+-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 soil. The ANI was attributed to, i. prolonged availability of NH4+-N; ii. enhanced mineralization of soil N and iii. greater root biomass.

The mechanisms responsible for added nitrogen interaction: Jenkinson et al. (1987) have described in detail the functional 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, 1967), ii) release of cellular components (Marumoto et al., 1977), iii) partial or complete denitrification followed by nitrification of 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 can occur simultaneously in the same soil. 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 soil N as a result of immobilized 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 decrease in fertilizer N applications. Similarly, a positive apparent ANI also causes fertilizer uptake efficiency to appear lower when measured by the uptake of 15N 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, 1988; 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 NH4+ and NO3− in causing an ANI is concerned since the former is reported to cause a higher ANI than the later. This means that added NH4+ gets exchanged with the native soil reservoir or bound NH4+. 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 not be mistaken as originating from the added nitrogen. Because of the recalcitrance of relatively stabilized soil N (bound N), NH4+ on the exchange complex or relatively labile biomass N will be more susceptible to such an exchange or displacement. However, the displacement reactions 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 NH4+ could be displaced from the soil NH4+ 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 15N-NH4+ and the native clay-fixed NH4+. They suggested that variations in NH4+ 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 NH4+ gets exchanged with that in the microbial biomass. Normally, however, upon entry into the microbial cells, NH4+ is incorporated almost instantly into the amide group of glutamine and into the major biochemical pathways. Hence extracellular NH4+ 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-mineralization reactions the applied NH4+ is 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 the apparent ANI. As described by Jenkinson (1986) defined pool substitution as the process by which labeled 15N added to a particular pool takes the place of unlabeled 15N soil N (or in other words stands proxy for the unlabeled soil N) that would otherwise have been abstracted from that pool. In order to occur it is essential that redox conditions in soil are conducive for a net immobilization, and ii) microbes discriminate 15N (added) against 15N (inert). The first condition is easily met in soils containing sufficient quantities of easily decomposable organic matter following addition of fresh manure 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 Brenner (1976) that no significant discrimination for 15N and 15N 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 principal for producing 15N-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 15N content due mainly to the preferential loss of nitrogen by processes like denitrification. It is this difference in 15N 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 ANI, then this fertilizer application 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, additional report to soil derived 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 NH₄⁺ is assimilated by the soil microorganisms in preference to NO₃⁻ (Jansson and Stanford, 1957) and Craswell and Strong (1976) observed very little immobilization of NO₃⁻ into N in fallow soils. Hence addition of NO₃⁻ to a soil immobilizing N is not expected to cause an ANI. Some other studies, however, not only show a net immobilization of NO₃⁻ into N, but a positive ANI as well (Azam et al., 1989, 1993b). Zagal and Persson (1994) also observed rapid immobilization of NO₃⁻ and a positive ANI in the presence of glucose. It follows therefore that immobilization-driven pool substitution cannot be the only process involved (Neleson and Kurz, 1973). Nevertheless, the occurrence of soil N immobilization 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 (Jenkins et al., 1986) following addition of labeled NO₃⁻. However, as mentioned earlier, ANI is generally more obvious following addition of NH₄⁺ rather than NO₃⁻; while addition of the former is reported to cause a higher denitrification of any NO₃⁻ added or that already present in the soil (Azam et al., 2001). This increase has been attributed to the development of anaerobic microorganisms, especially in the presence of NH₄⁺ that enables microbial proliferation more than NO₃⁻. Under these circumstances, ANI will be negative rather than a positive apparent ANI. Nevertheless, a positive apparent ANI in NO₃⁻ 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 NH₄⁺ salts to be more effective in stimulating the mineralization rates in the presence of NH₄⁺ than either of NH₄⁺ or NO₃⁻. Jenkins et al. (1955) persist with the idea that large ANIs observed in case of NH₄⁺ are largely apparent although no real sound evidence is available to support this contention. Studies do show, however, that mineralization of organic N is enhanced by added fertilizer N especially NH₄⁺ (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 Jenkins et al. (1965) becomes questionable. Hence, any increase in the extractability, mineralization, and plant availability of unlabelled N from soil following application of labeled N (or vice versa) should be considered real that results from the changes in the processes that move N into or out of a particular compartment as aptly defined by Jenkinson et al. (1986). 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 CO₂ and consumption of O₂. Usually the net mineralization is either the same or lower in 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 CO₂ production in field soils (De Jong et al., 1974). Kowalski et al. (1978) also showed that incubated samples of N fertilized soil consumed less O₂ than did samples of control soils. This suggests that mineral N is utilized more rapidly by the soil microorganisms than by the added fertilizer N. Even 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 NH₄⁺-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 ¹⁵N-labelled fertilizers. This enhancement is evident from the fact that use efficiency of fertilizer N by non-isotopic difference method is generally higher as compared to that by 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., 1986) 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 (Sorensen, 1982). Some other studies, however, show that root biomass may not necessarily be responsible for enhanced N uptake as pruned and restricted roots may also 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 N (Krämer et al., 1987; Zagal, 1994; Kuzjakov, 2000a). According to Kuzjakov (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 by the ANI. Contributions of each of these contributing factors to 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 NO₃⁻ leaching may become an important manifestation of ANI (Kuzjakov et al., 2000b; Barcelough et al., 1984; Wu et al., 1991). Added N, particularly NH₄⁺ may also enhance the loss of NO₃⁻ 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 high C availability, removal of native soil NO₃⁻N through immobilization and denitrification may lead to a negative ANI. Application of NH₄⁺ or NH₄⁺-forming fertilizers will lead to a higher ANI than NO₃⁻. In most cases, the ANI is real and caused by ii increased microbial activities, especially the process of mineralization-immobilization turnover and iii increased root activity enabling the plants to
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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.

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