SOIL BIOLOGY AND BIOCHEMISTRY

Anna K. Kvitkina, Alla A. Larionova, Sergei S. Bykhovets

EFFECT OF EXTERNAL AND INTERNAL NITROGEN ON MINERALIZATION RATE OF CORN RESIDUES

Institute of Physicochemical and Biological Problems in Soil Science of RAS (IPCBPSS RAS), Pushchino, Russia, aqvia@mail.ru. Tel : +89269336403

Abstract. The impact of external and internal nitrogen on plant residues decomposition was studied in a series of long-term laboratory incubation experiments. Experiment A was conducted with corn leaves with variable C:N ratios 22, 34, 47 and 62. C:N ratios in Experiments B and C were adjusted to 47, 32, 22 and 10 by adding NH_4NO_3 (Experiment B) or KNO_3 (Experiment C) to corn residues with initial C:N ratio of 62. Mineralization rates of labile and recalcitrant carbon pools of plant residues were estimated by kinetics of cumulative CO_2 losses during one-year-long incubation simulated by the double exponential decay function. The internal organic nitrogen was shown to effect the decay constant of the labile pool only, while the internal inorganic N had impact on the labile pool size as well. Also, KNO_3 as an external N form influenced both k_1 value and the labile pool size. NH_4NO_3 affected all the parameters of the double exponential decay model including second decay constant (k_2) of recalcitrant pool. Thus, mineralization of plant residues depends significantly both on concentration and form of available nitrogen.

Keywords: CO_2, mineralization constant, C:N, litter quality, external and internal nitrogen.

INTRODUCTION

The carbon-to-nitrogen ratio (C/N) is the key index of the litter quality which is widely used to predict litter decomposition dynamics. Recently anthropogenic activity has increased nitrogen deposition in ecosystems and decreased the C/N ratio in litter (Gruber and Galloway, 2008). However, it is still discussed how the nitrogen affects the litter decay: does it inhibit or stimulate the mineralization?

Decomposition rates are affected by the chemical, physical and biological factors of the environment, as well as the quality of the litter: nitrogen, Ca, lignin and polyphenols concentrations, C/N and lignin to N ratio (Larionova et al., 1995, Semenov et al., 2004, Knorr et al., 2005, Berg, Meentemeyer, 2008, Zhang et al., 2008, Hobbie et al., 2012). Advanced data are contradictory: there are evidences that nitrogen can both increase (Blagodatsky et al., 1993, Enriquez et al., 1993, Hobbie, 2000, Zhang et al., 2008, Cornwell et al., 2008, Weedon et al., 2009) and suppress (Bowden et al., 2004, Zak et al., 2011, Thomas et al., 2012) litter decomposition. Effect of nitrogen addition varies between labile and stable, low-lignin and high-lignin components. Nitrogen fertilization stimulates the decomposition rate of the low-lignin litter significantly more than that of the high-lignin litter (Hobbie, 2000). N deposition inhibits lignin decomposition through the repression of phenoloxidase and peroxidase in basidiomycotes which mineralize lignin (Zak et al., 2011). N stimulated the decomposition at the initial stages, while at the later stages N addition resulted in SOM stabilization and decreased soil respiration (Berg and Meentemeyer, 2008).

We assume that along with the quality of the carbon compounds (Kogel-Knabner, 2002), nitrogen source affects the plant residues mineralization as well. Decomposition rates are expected to differ depending on offer internal N concentration and external nitrogen applied as fertilizer or depositions. Earlier response of litter decay rates and microbial community to multiply forms of nitrogen enrichment were studied in sandy forest soils (Hobbie et al., 2012). On results, both substrate N and externally supplied N evaluated the initial decomposition rate, which corresponded to higher activity of polysaccharide-degrading enzymes. Later externally supplied N slowed decay rates, increasing the slow fraction of litter and reducing lignin-degradative activity. The aim of this study was to compare the impact of mineral and organic nitrogen sources on litter de-

composition. The hypothesis was that the effect of added (external) mineral nitrogen vs. nitrogen inherited in plants (internal) on litter decay are not equal.

MATERIAL AND METHODS

Experimental Design

Corn (Zea mays L.) was grown in sand-filled pots at 27/15°C day/night temperature, with an atmospheric humidity of 80 – 85 % and 16 h photoperiod. The N treatments were as follows: poor (from 0 to 8.4 mg N/kg of sand), moderate (84 mg N/kg of sand) and rich in N (from 168 to 672 mg N/kg of sand). Moderate N fertilization rate was equivalent to that in Pryanishnikov mixture: 0.24 g NH_4NO_3 per kg of sand (Practical guide ..., 2001). Other nutrients were applied at the same rate for all the N treatments as in the Pryanishnikov mixture. Plants were harvested 2 months after emergence at jointing stage (7 leaves), then we divided plant biomass into roots, green leaves, yellow wilted leaves, stems, dried at 25°C and milled it. C:N ratio in the yellow wilted corn leaves (hereinafter - corn leaves) was 62, 47, 34, 22 depending on the nitrogen fertilization rate.

The corn leaves were incubated in the microcosm (Pestryakov et al., 1990) in experiments A, B, C at 22°C, 80 % WHC (water holding capacity of dried leaves was approx. 300 %). Each sample consisted of milled corn leaves (100 mg), mixture of HCl-treated-sand (900 mg) with illite (100 mg). Samples in 5 replicates were placed into 13 ml sealed vials and inoculated with 100 µl of 10:1 water: chernozem soil suspension and incubated during 365 d. In experiment A corn leaves with C:N ratios 22, 34, 47, and 62 were incubated without N addition (treatments 22A, 32A, 47A, and 62A). In experiments B and C we adjusted corn leaves with C:N 62 to C:N 47, 32, 22, and 10 by adding NH4NO3 (Experiment B, treatments 47B, 32B, 22B and 10B) and to C:N 47 and 22 by adding KNO_3 (Experiment C, treatments 47C, 22C). In total there were 50 samples (10 variants by 5 replicates). On 50 day, on 200 day and 365 day single replicate was seized to determine Corg and Ntot amount.

CO_2 Emission

Gas samples were taken at 1, 3, 5, 7, 10 day, then weekly or less within 365 days of the experiment, after gas sampling vials were ventilated and sealed. The concentration of CO_2 was determined by gas chromatograph «Cristallucks»-4000 with a thermal conductivity detector. The gas mixture was separated on a column of 3 m length, filled with sorbent Porapack-Q, at 50°C.

The respiration rate was calculated as the accumulation of CO_2 in the intervals between the gas sampling. Mineralization of the organic substance (C_{min}) was determined as the cumulative emissions of carbon dioxide (C-CO_2), calculated as a percentage of the initial carbon (C_0):

$$C_{min} = C\text{-}CO_2/C_0 100\ \% \qquad (1)$$

Carbon and Nitrogen Content

Organic carbon (Corg) and total nitrogen (Ntot) content was determined by HCNS-elemental analyzer Vario EL III («Elementar», Germany) in milled samples. Amount of ammonia (N-NH_4) and total inorganic nitrogen (Ninorg) was determined in the water extract (100 mg dry leaves per 40 mL of water), with the colorimetric method using phenol hypochlorite reaction (Kudeyarov V.N. 1965). Amount of nitrate-N (N-NO_3) was calculated as Ninorg minus N-NH_4. Concentration of organic nitrogen (Norg) in plant tissues was calculated as Ntot minus Ninorg.

Data Analysis

In our experiment mineralization of corn residues in the microcosm was best described by double exponential model (Equation 2):

$$C_{\min} = 1 - A_1 e^{-k_1 t} - (1 - A_1) e^{-k_2 t} \qquad (2)$$

where A_1 – size of the labile pool, k_1 and k_2 - rate constants of the labile and recalcitrant

carbon pool, respectively. Equation was approximated using Marquardt algorithm.

Mean residence time was calculated as the reciprocal of the decomposition rate constant:

$$MRT = 1/k \quad (3)$$

Statistical Analysis

Data were analyzed by the program Statistica 6.0. All data were expressed as the mean of three replicates ± Standard deviation, except for the first 30 days of incubation (five replicates ± Standard deviation). A posteriori groups comparison were analyzed using Tukey test ($P<0.01$). Two-way ANOVA was used to test decomposition rates at different time intervals in each experiment separately ($P<0.01$). Three-way ANOVA was used to test the effects of C/N (C/N 47 and 22), the nitrogen source (internal N, external NH_4NO_3 and external KNO_3) and sampling date (7 d, 90 d, 365 d) on cumulative CO_2 release ($P<0.01$).

RESULTS

Organic and Inorganic Nitrogen Content in the Corn Leaves

Internal nitrogen was presented by organic (Norg) and both inorganic (Ninorg) (ammonia and nitrate) forms (figure 1). Higher rates of N fertilization during the plant growth resulted in increase of all N forms content in plant biomass. The most prominent effect was for nitrate, with an increase in NO_3-N content from 0.04 % to 0.94 %, followed by ammonia (0.008 % to 0.033 %) and organic N (0.572 % to 0.982 %). Also, litters of plants grown at different N fertilization rates demonstrated different C:N and organic N- to -mineral N ratios. As C:N in leaves decreased from 62 to 22, Norg:Ninorg declined from 12 to 1; in other words, corn leaves with C:N 22 contained equal amounts of organic and inorganic nitrogen. C:N ratios in plant material in the experiments B and C were adjusted to the appropriate values as in the experiment A by adding external nitrogen.

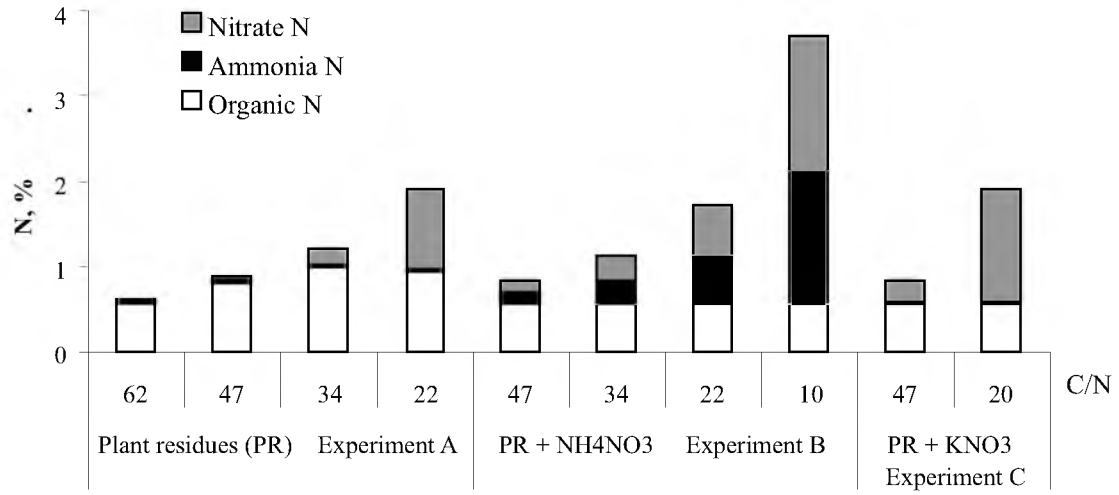

Figure 1 - The content of organic, ammonia and nitrate nitrogen in plant residues (PR), % of dry biomass

Decomposition Dynamics of Corn Leaves

The highest rates of CO_2 emission were observed within the first 20 days of incubation (figure 2) when more than half of organic carbon (50-80 %) was mineralized.

During the early stage (days 0–7), the maximum decomposition rate of corn leaves with C:N 62 was 1.2 % Corg per day while that for leaves with C:N 22 was as high as 2.8 % (experiment A). N addition increased the de-

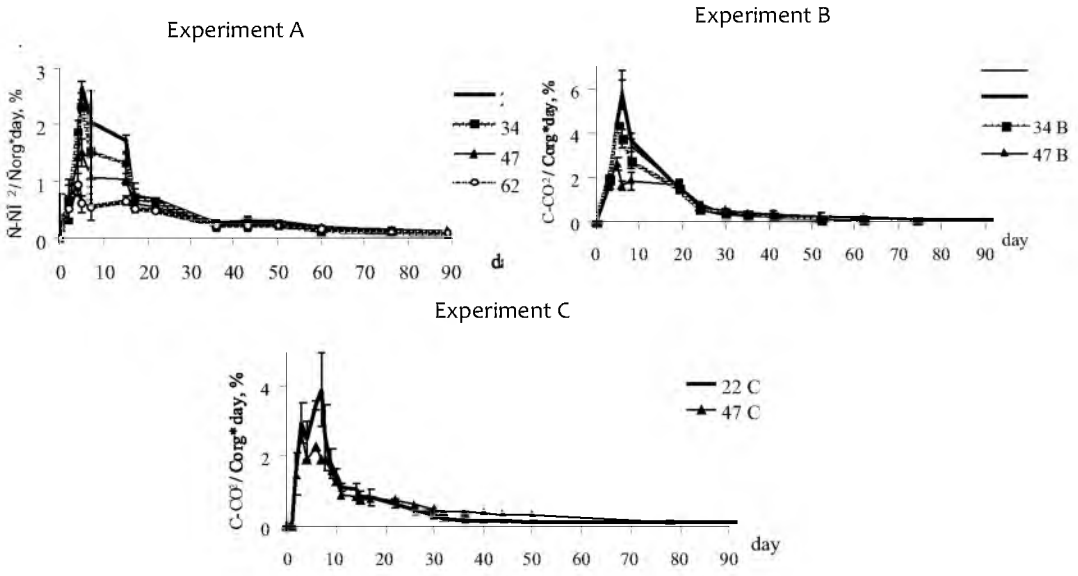

Figure 2 - Rates of the CO_2-emission, as % of the Corg per day, by decay of plant residues (experiment A), NH_4NO_3 treatment (experiment B), KNO_3 treatment (experiment C) for 90 days

cay rate to 5.0 % and 7.1 % Corg per day at C:N 22 and C:N 10 for KNO_3 and NH_4NO_3, respectively (figure 2). After 20 days of incubation, the decomposition slowed down, with maximum rate of 1.7 % Corg per day in NH_4NO_3 treatment followed by 0.4-1.0 % and 0.5-0.9 % Corg per day in experiments A and C, respectively. After 30 days of incubation, the decomposition rates decreased by one order of magnitude and did not differ significantly ($P < 0.01$) between the experiments anymore.

Effect of Nitrogen on Cumulative Carbon Losses at Different Time Intervals

Mineralization of corn residues depended both on C:N ratios and N source ($P < 0.01$). The greatest effect of nitrogen rates on cumulative carbon losses was found at the initial stages of decomposition (days 0–7) (figure 3).

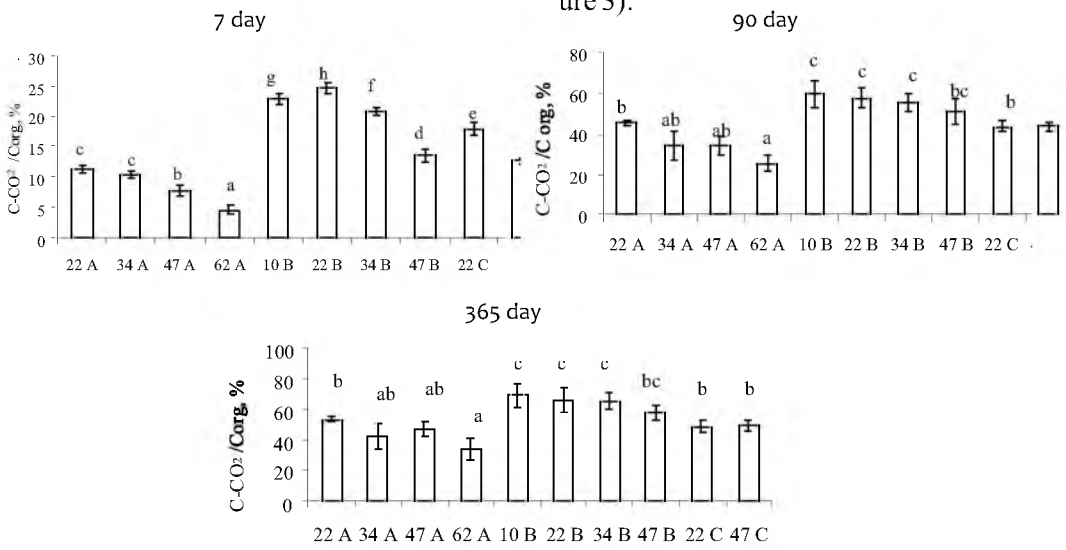

Figure 3 - Cumulative carbon losses during the corn residues decomposition for 7, 90 and 365 days of incubation. Indices (a-h) show statistically significant differences between the variants ($P < 0.01$)

The C:N effect decreased with time irrespective of N source (internal N, external NH_4NO_3 or external KNO_3). Therefore treatments with different nitrogen concentrations resulted in similar cumulative carbon losses by day 365. The low concentrations of external nitrogen and the large concentrations of internal N resulted in the same CO_2 emissions. Namely, 7 day cumulative carbon losses for the variants 22A and 47C did not differ significantly ($P < 0.01$). Cumulative carbon emission between the variants 47B, 47C and 22A ($P < 0.01$) were not significantly different from 90 days until the end of the experiment (figure 3). By the end of incubation (day 365 d) 33.5 % of corn leaves with C:N 62 were decomposed. At C:N 22 the percentage of decomposed C enhanced to 53.1 %, 48,4 % and 65,9 % for the 22A, 22C and 22B treatments.

Nitrogen Effect on the Parameters of the Double Exponential Decay Function

N source had a significant impact on the size of labile pool A_1: application of both ammonium and nitrate forms of external nitrogen (NH_4NO_3 treatment) led to an increase of the labile pool A_1 by 1.9-2.2 times as compared to internal organic nitrogen treatment (figure 4a), whereas the presence of the external or internal nitrate enhanced A_1 size only by 1.6 times.

C:N shift from 62 to 22 resulted in significant increase in labile pool from 26 % to 42 % Corg (figure 4a), due to the equal content of mineral and organic internal nitrogen in 22A.

Internal and external N affected not only the labile pool size (A_1), but its decay constant (k_1) as well. C:N decrease by both internal and external nitrogen amendments resulted in an increase of k_1 by 1.6-2.4 times (figure 4b). Depending on the rate of N addition, mean residence time of labile pool varied from 14 to 31 days.

In contrast to A_1 and k_1, values of second decay constant (k_2) of recalcitrant carbon pool were independent on C:N ratio, except those for NH_4NO_3 treatments (figure 4b). Addition of NH_4NO_3 accelerated mineralization of recalcitrant pool by 3.3 times, with decreasing MRT from 8.7 to 2.7 years.

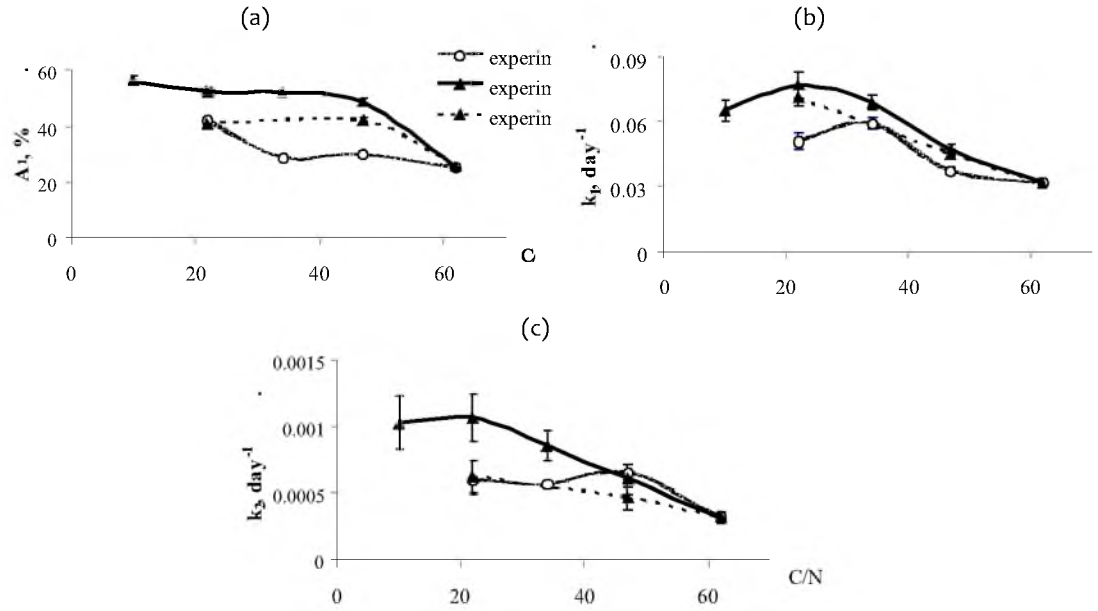

Figure 4 - Parameters of the double exponential decay model of corn residues: (a) A1 - the size of the labile pool (% of Corg), (b) k1 - constant of the labile pool mineralization, d-1, (c) k2 - constant of the recalcitrant pool mineralization, d-1, Variants: experiment A - plant residues, experiment B – plant residues + NH_4NO_3, experiment C - plant residues + KNO_3

DISSCUSSION

Effect of Nitrogen on Cumulative Carbon Losses at Different Time Intervals

Enhancement of both internal and external N content led to increase of cumulative carbon losses. Cumulative losses differed dramatically in the period of 0 - 20 days, with maximal C:N impact during the first 7 days. It agrees well with data by Zhang et al. (2014); the authors reported significantly different effect of $NH4^+$ and NO_3 on litter decay rates in early mineralization stage (days 0 – 15). Also, decline in C:N effect with time was found in similar laboratory and field experiments. This is in agreement with results obtained by Moran et al. (2005), showing that total soil respiration was higher with inorganic N-treatment, although the difference was significant ($P < 0.05$) only in first 60 days.

According to other authors (Semenov et al., 2004), C:N ratio affected litter mineralization during 56 d, and later the cumulative carbon losses were not dependent on C:N. In contrast to labile pool nitrogen addition led to the accumulation of slowly decomposing organic matter pool subsequently (Hobbie et al., 2012, Zhang et al., 2014). This can be explained by changes in biochemical properties of litter and shifts in its exoenzymes activity due to inorganic N, which increased the activity of hydrolytic enzymes that degrade polysaccharides, but reduced the activity of lignin-decomposing phenoloxidases (Hobbie et al., 2012, Thomas et al., 2012).

Eventually, NH_4NO_3 addition caused the maximum litter mineralization. In contrast to our results, Zhang et al. (2014) reported that NO_3^- addition to soil enhanced litter mineralization greater than NH_4^+ addition.

Nitrogen Effect on the Parameters of the Double Exponential Decay Function

Our results show that external nitrogen accelerates decomposition more intensively than internal. Addition of small amount of external mineral nitrogen in situ led to the same carbon losses as high concentrations of internal nitrogen in corn leaves (figure 3). Differences in the impact of internal vs. external nitrogen should be reflected in the mathematical models simulating decomposition of soil organic matter, since N source affects parameters of exponential models.

Internal organic nitrogen increased only the rate of decomposition of the labile pool (k_1). The size of the labile pool was inversely related to C:N ratio in corn residues and total internal nitrogen. Our results are in accordance with data by Hobbie et al. (2012), wherein the leaves with an initially high amount of nitrogen pool contained greater labile carbon pool vs. low nitrogen leaves. Addition of external nitrate nitrogen increased both the rate of decomposition k_1, and the size of the labile pool (A_1). NH_4NO_3 deposition, along with the A_1 and k_1, increased decay constant for recalcitrant carbon pool (k_2) as well.

The differences between internal and external N forms we explained by their contrast roles in the nitrogen transformation cycle. External nitrogen enters the microcosm in completely available inorganic form that corresponds to the active fraction of inorganic soil nitrogen (Semenov et al., 2001). Increase of inorganic N resulted in its rapid immobilization and growth of microbial biomass, i.e. intensified utilization of available C. Our data on higher decomposition rates after application of inorganic N (figure 2) seem to support this hypothesis. By contrast, internal organic N is being exposed first to mineralization-immobilization cycling, including depolymerization of plant proteins, amino acids mineralization and nitrogen immobilization by microbial community. Nitrogen assimilation by microorganisms coincides with a fast stage of plant residues decomposition, but its quantity is limited: only 11.7 % and 19.8 % of roots nitrogen (C:N 99) and the above-ground nitrogen (C:N 55), respectively, were immobilized by microbial bio-

mass in first 5 days of corn residues incubation (Semenov et al., 2001). At the later stages of decomposition nitrogen assimilation by microorganisms was compensated by its mineralization (Semenov et al., 2001).

Differences in decay rates at the presence of ammonium vs. nitrate may be attributed to unequal hydrolytic activity of enzymes and microbial growth rate. As the ammonium and nitrate N are quite rapidly, within a few days, immobilized by microorganisms, ammonia nitrogen is more favorable for soil microorganisms (Merrick, Edwards, 1995). Ammonia nitrogen is directly involved into the synthesis of amino acids and proteins, and promotes rapid growth of microorganisms and activation of enzymes that break down polymeric carbon compounds. Unlike ammonia, nitrate nitrogen is exposed to reduction reactions before being involved into protein synthesis. Nitrate reduction process occurs in the presence of labile carbon compounds. Thus, a portion of labile C pool is utilized on nitrate reduction that reduces microbial growth. Also, there are losses of nitrogen in gaseous form during dissimilatory nitrate reduction (denitrification). Therefore addition of external nitrate alone can result in a decrease of microbial biomass, which would eventually become less active due to nitrogen deficiency after N leaching and gaseous losses during denitrification.

Thus, the nitrogen source and the form in which it is included into litter decomposition (organic, nitrate, ammonium) affect the carbon decay constants and labile pool size. Therefore we suggest that the models of the carbon cycle should simulate N addition effect separately for each N form.

CONCLUSION

C:N ratio, either in plant residues, or in situ, affects litter decomposition. CO_2 release is strongly affected by C:N during early stages of decomposition (days 0-20), when C:N decrease resulted in higher decomposition rates. Effect of the nitrogen source (internal N, external NH_4NO_3 and external KNO_3) did not change with time, while that of C:N ratio decreased. Partitioning internal N pool between organic and inorganic N forms also affects plant residues decomposition.

Also, C:N affects the parameters of the double exponential model of plant decomposition. Decay constants of labile carbon pool are directly dependent on C:N in the range of 22- 62, with decreasing impact at high rates of external (C:N 10) and internal (C:N 22) nitrogen. Litter decomposition depends not only on C:N ratio, but on the source and form of available nitrogen as well. Internal and external nitrogen, added as KNO_3 or NH_4NO_3, act in the same direction, but with different intensity: the effect of external nitrogen is stronger than that of internal; the impact of ammonium is more pronounced than that of nitrate. Increase of internal organic nitrogen content accelerates the decay rate constant of labile carbon pool (k1) only. External nitrate nitrogen increases both the labile carbon pool (A_1) and k_1. Addition of NH_4NO_3 affects all the parameters of the model describing the degradation of plant residues, including second decay constant (k_2) of recalcitrant pool.

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РЕЗЮМЕ

А.К. Квиткина, А.А. Ларионова, С.С. Быховец

ВЛИЯНИЕ ВНЕШНЕГО И ВНУТРЕННЕГО АЗОТА НА СТЕПЕНЬ МИНЕРАЛИЗАЦИИ ОСТАТКОВ КУКУРУЗЫ

Институт физико-химических и биологических проблем почвоведения РАН (IPCBPSS РАН), Пущино, Россия, aqvia@mail.ru. Тел : +89269336403

Влияние внешнего и внутреннего азота на разложение растительных остатков было изучено в ходе серии долгосрочных лабораторных экспериментов инкубации. Эксперимент А проводили с листьями кукурузы с переменным соотношением C:N -22, 34, 47 и 62. Коэффициенты C:N в экспериментах В и С доводили до 47, 32, 22 и 10, путем добавления NH_4NO_3 (эксперимент Б) или KNO_3 (эксперимент С) в остатки кукурузы с исходным соотношением C:N 62. Уровень минерализации лабильных и устойчивых углеродных резервов растительных остатков оценивались при помощи кинетики кумулятивных потерь CO_2 в течение однолетней инкубации, моделируемой с помощью функции двойного экспоненциального распада. Внутренний органический азот показал, что влияет только на константу распада лабильного резерва, в то время как внутренний неорганический N влияет также на размер лабильного резерва. Кроме того, KNO_3 в качестве внешней формы N влиял как на значение k_1, так и на размер лабильного резерва. NH_4NO_3 повлиял на все параметры модели двойного экспоненциального распада, в том числе второй константы распада (k_2) устойчивого слоя. Таким образом, минерализация растительных остатков в значительной степени зависит как от концентрации так и от формы доступного азота.

Ключевые слова: CO_2, постоянное значение минерализации, C: N, качество остатков, внешний и внутренний азот

ТҮЙІН

А.К. Квиткина, А.А. Ларионова, С.С. Быховец

ІШКІ ЖӘНЕ СЫРТҚЫ АЗОТТЫҢ ЖҮГЕРІ ҚАЛДЫҚТАРЫНЫҢ МИНЕРАЛДАРМЕН ҚҰНАРЛАНУЫНА ӘСЕР ЕТУІ

РҒА топырақтану физика-химиялық және биологиялық мәселелер институты (IPCBPSS РҒА), Пущино, Ресей, aqvia@mail.ru. Тел : +89269336403

Ішкі және сыртқы азоттың өсімдік қалдықтарының ыдырауына әсер етуі ұзақ мерзімдік зертханалық инкубация тәжірибелері барысында зерттелді. А тәжірибесі C:N - 22, 34, 47 және 62 ауыспалы арақатынасымен жүгері жапырақтарына жүргізілді. В және С тәжірибелерінде C:N коэффициенттері C:N 62 бастапқы арақатынаспен жүгері қалдықтарына NH_4NO_3 (Б тәжірибе) немесе KNO_3 (С тәжірибе) қосу арқылы 47, 32, 22 және 10-ға жеткізілді. Өсімдік қалдықтарының тұрақсыз және тұрақты көміртек қорларын минералдармен құнарландыру деңгейі қосарлы экспоненттік ыдырау қызметінің көмегімен үлгілендірілетін бір жылдық инкубация ішінде CO_2 кумулятивтік шығын кинетикасының көмегімен бағаланды. Ішкі органикалық емес N тұрақсыз шама мөлшеріне де әсер етсе, ішкі органикалық азот тұрақсыз шама ыдырауының константына ғана ықпал ететінін көрсетті. Сонымен қатар, KNO_3 сыртқы пішін N ретінде k_1 мәніне де, NH_4NO_3 тұрақсыз шама мөлшеріне де, қосарлы экспоненттік ыдырау үлгісінің барлық өлшемдеріне, оның ішінде тұрақты қабат ыдырауының екінші константына (k_2) ықпал етті.

Осылайша, өсімдік қалдықтарының минералдармен құнарлануы азоттың шоғырлануынада, оның түріне де байланысты болады.

Кілтті сөздер: CO_2, минералдармен құнарланудың тұрақты мәні, C: N, қалдықтардың сапасы, ішкі және сыртқы азотCO_2, постоянное значение минерализации, C: N, качество остатков, внешний и внутренний азот