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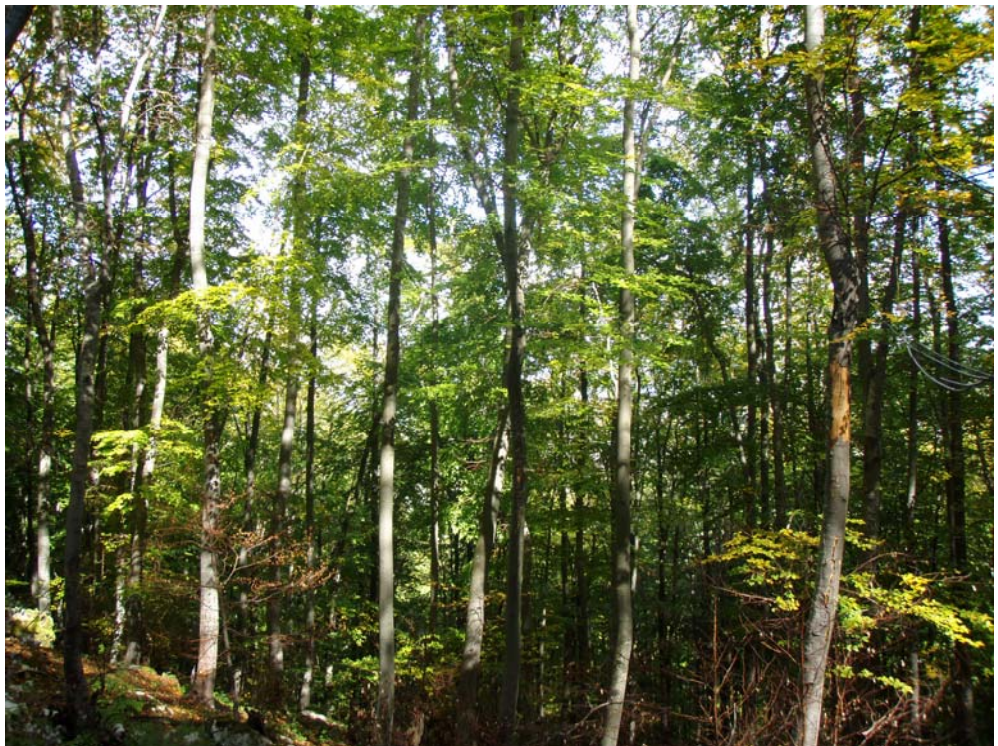


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SUSTAINABLE FORESTRY ODRŽIVO ŠUMARSTVO

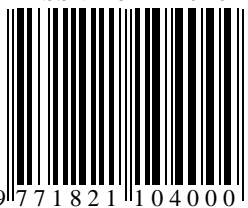
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**THE MICROORGANISMS POPULATION SIZE OF THE SOIL
ORGANIC LAYER AND CHARACTERISTICS OF THE
LITTERFALL OF THE COMMON ALDER (*ALNUS GLUTINOSA*
GAERTN.) ON THE RECLAIMED MINE SOIL OF MINING-
ENERGY-INDUSTRIAL COMPLEX "KOLUBARA"**

*Zoran MILETIĆ¹, Bratislav MATOVIĆ¹, Zoran PODUŠKA¹,
Zlatan RADULOVIĆ¹, Snežana STAJIĆ¹, Vlado ČOKEŠA¹*

Abstract: *The researches were conducted in three common alder monocultures, established by the deposition of the waste-rock from the open-pits of the lignite mines. The total annual yield of the litterfall, organic carbon and nitrogen, phosphorous, and potassium was studied. The ratio C/N and the hydrolyzed nitrogen forms were determined. In the organic soil layers and surface layers of soil covered by the common alder the seasonal dynamics of the number of the saprophytic microorganisms, which have the important role in the nitrogen cycle in the forest ecosystems, was studied.*

Key words: common alder, litterfall, organic soil layer, saprophytic microorganisms, plant assimilatives.

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BIOGENOST ORGANSKE PROSTIRKE I KARAKTERISTIKE LISNOG OPADA CRNE JOVE (*ALNUS GLUTINOSA* GAERTN.) NA DEPOSOLIMA REIK "KOLUBARA"

Izvod: Istraživanja su izvršena u tri monokulture crne jove, podignutim na deposolima nastalim odlaganjem jalovine sa površinskih kopova rudnika lignita. Ispitivan je ukupan godišnji priliv lisnog opada, organskog ugljenika i azota, fosfora i kalijuma. Određen je C/N odnos i hidrolizujući oblici azota. U organskoj prostirci i površinskim slojevima zemljišta pod kulturom jove ispitana je sezonska dinamika brojnosti saprofitnih mikroorganizama koji obavljaju važnu funkciju u kruženju azota u šumskim ekosistemima.

Ključne reči: Crna jova, lisni opad, organska prostirka, saprofitni mikroorganizmi, biljni asimilativi

1. INTRODUCTION

The reclaimed mine soils formed by the deposition of the waste-rock from the open-pits of the lignite mines in the Kolubara basin have a deep solum, and in regard to the deposition patterns are the mosaics of the different lithologic layers of the different texture and mineralogical composition. The physical and chemical, as well as microbial characteristics of these reclaimed mine soils were studied by numerous authors (Stojanovic et al. 1977; Antonovic et al. 1978; Antonovic et al. 1984; Veselinovic et al. 1984; Rasulic 1997; Schmidt, Veselinovic 1997; Schmidt, Miletic 1997, Drazic 1997; Miletic 2004, Miletic, Radulovic 1995). All these authors mention that the greatest problems are posed by the unfavourable chemical characteristics of the anthropogenic soils formed in this way. It mainly refers to the complete lack of humus and nutrients, which also implies the complete lack of organic nitrogen forms, from which the forms that are available to the plants are released. For the majority of the oligotrophic forest species the poor supply with the phosphorous and potassium forms, which are available to the plants, poses a smaller problem, since these elements are still present in the available forms. The source of these forms is parent rock, and under the influence of the agents of the mineral decomposition on the soil surface these elements which are unavailable to the plants turn into the forms which are available to the plants.

The growth and development of growth of the forest trees, which also means the production of wood volume, depend upon the nutrition of the forest trees. The main limiting factor for the growth and development of the forest trees on the reclaimed mine soils of the Mining-Energy-Industrial Complex "Kolubara" is the lack of nitrogen in the soil.

The effectiveness of the reforestation depends upon the applied reforestation technology, i.e. upon the used organic fertilizers. It mainly refers to peat, which is introduced to the planting pits during the reforestation, and by which the certain quantity of the organic nitrogen is also introduced. The further nutrition of the forest trees by nitrogen depends on the established balance of the cycle of this element in the artificially established ecosystem. During the early years of the development of the forest plantations the quantity of the nitrogen which circulates in the artificially established ecosystem is small.

The forest species which can use the atmospheric nitrogen by the symbiotic nitrogen fixation do not have any difficulties in the nutrition by nitrogen. The litterfall of the trees which use the atmospheric nitrogen is always rich in this element of nutrition, which is deficient on the reclaimed mine soils. By the processes of the biodegradation of the organic nitrogen (amination, ammonification, nitrification, and nitration) under the influence of the saprophytic microorganisms the organic nitrogen forms convert into the mineral forms NH_4^+ and NO_3^- (Savic, Jekic 1975), which are available to the plants, and can be used by the species which cannot use the atmospheric nitrogen by the symbiotic nitrogen fixation.

The common alder is the species with the ability of the symbiotic nitrogen fixation (Blom et al. 1981). This paper is aimed at the study of the possibilities of the use of the common alder in the mixed cultures, which by the production of the litterfall rich in the nitrogen could improve the nutrition of the other, more economically beneficial tree species by this missing element in the soil.

2. METHODS

The characteristics of the litterfall of the common alder aged 11, 15, and 16, were studied on the reclaimed mine soils of the Mining-Energy-Industrial Complex "Kolubara". Only the newly-formed litterfall, which was transported to the soil surface in the year of the research, was studied. In order to prevent the mixing of the one-year-old litterfall with the organic soil organic layer i.e. the mixing with the older litter, the litterfall was collected in the wooden boxes, which were introduced in the plantations prior to the phenophase of the leaf loss.

In the litterfall collected in this way by the analysis of the ash obtained by the dry burning the following macro elements of the nutrition were determined: the total phosphorous colorimetrically, the total potassium flame-photometrically, the total calcium and magnesium complexometrically (Dzamic et al. 1996). The content of the total nitrogen was determined by the use of Kjeldahl's method, and the content of the total nitrogen by Anstet's method,

modified by Punomarjeva and Nikolajeva (Ponomareva, Plotnikova 1975). From the hydrolyzed nitrogen forms the total hydrolyzed, ammonium, amino-acidified, and hexosamine nitrogen forms (Black et al. 1965) were determined.

In the horizon of the organic soil layer the number of the physiological soil microorganisms, which have a role in the dynamics and balance of the nitrogen cycle in the forest ecosystems, was determined. The number of the ammonifying microorganisms on MPA, the total number of the microorganisms on the soil agar and the number of free nitrogen fixing on Krasilnikov's agar were determined.

3. RESULTS AND DISCUSSION

The litterfall is the most important source of the organic carbon for the forest soils. Along with the litterfall, nitrogen and other nutrients are transported to the soil surface (Rodin, Bazilevič 1965; Nordén 1994). The production of the litterfall on the soil surface under the common alder cultures is high. The measured quantities of the litterfall under the common alder monocultures range from 1,775.50 to 2,612.5 kg of the completely dry nutrients per a hectare. This wide range of the produced litterfall on the soil surface is the result of site and stand conditions.

In contrast to the total quantity of the common alder litterfall, the chemical characteristics of it are much more equalized. The content of the total nitrogen in the litterfall is 2.4 %, and its annual yield on the soil surface under the monocultures is 51.57 kg/ha (Table 1). This quantity of nitrogen is in the harmony with the quantity of the mineral fertilizer NPK 15:15:15 - 343.8 kg. The mineralisation of the organic nitrogen in the oxidative environment occurs as the oxidation process, and in the anoxidative as the hydrolytic process (Tescic, Todorovic 1997).

The greatest part of the total nitrogen from the common alder litterfall is not subject to hydrolysis, i.e. the oxidative conditions are needed for the mineralisation of it. There is also a great quantity of hydrolyzed nitrogen which can also decompose in the anaerobic conditions. The amino acid nitrogen is the most common form of the hydrolyzed nitrogen, and it is followed by ammoniacal nitrogen. The ratio of carbon to nitrogen is very narrow, i.e. 12.72. It is the precondition for the fast mineralisation of the nutrient and the conversion of all organically-bound plant assimilatives from the soil organic layer into the mineral forms, as well into the forms which are available to the plants. The annual quantity of total nitrogen which common alder plantations transports by the litterfall to the soil in the research area is higher than the quantity transported by black locust (Miletic 2004), which is also the species with the ability to use the atmospheric nitrogen. In addition, the ratio of carbon

to nitrogen of the common alder litterfall is narrower in comparison with the black locust litterfall.

Table 1. *The characteristics of the litterfall of the common alder*

| | % in litterfall | kg/ha | | % in litterfall | kg/ha |
|--------------------|-----------------|---------|-------------------------------------|-----------------|--------|
| Nutrient | 90.47 | 1987.30 | Ash | 9.53 | 206.70 |
| Total C | 30.25 | 659.79 | Total K ₂ O | 3.08 | 69.82 |
| Total N | 2.40 | 51.57 | Total P ₂ O ₅ | 0.21 | 4.57 |
| Hydrolyzed N | 0.87 | 19.21 | Total CaO | 4.03 | 87.43 |
| NH ₄ -N | 0.22 | 4.95 | Total MgO | 1.68 | 37.88 |
| Hexosamine - N | 0.07 | 1.41 | C/N = 12.72 | | |
| Amino acid -N | 0.42 | 9.63 | | | |
| Non-hydrolyzed N | 1.54 | 32.36 | | | |

Besides the high quantity of nitrogen, the common alder leaves contain the high quantities of the other macro elements of the nutrition. By the production of the litterfall the common alder transports the greater quantities potassium to the researched reclaimed mine soils. The quantity of potassium which the common alder monocultures annually produce on the soil is greater in comparison with the quantity of potassium under the linden and black locust plantations, and considerably greater in comparison with the quantities under the conifer species plantations (Miletic 2004). The studied plantations averagely transport 69.8 kg K₂O/ha on the soil surface. The common alder averagely transport 4.57 kg/ha of phosphorous, 87.43 kg/ha of calcium, and 37.88 kg/ha of magnesium to the soil surface by the leaf litter.

The great quantity of the litterfall produced by the common alder forms the horizon of the soil organic layer, which is the strongest in the autumn, after the phenophase of the leaf fall (O1 sub-horizon). From the next spring the organic soil layer is subject to the rapid mineralisation, as a result of the narrow ratio of carbon to nitrogen. In the autumn period, at the beginning of phenophase of the leaf fall, the soil organic layer is interrupted, and mainly made of the plant material produced by the other, native species. The coarse wooden fragments of the soil organic layer are also present, and to a lesser extent the common alder leaves as well.

In the spring the number of the amonifying microorganisms in the soil organic layer, and in surface soil, which is 2 cm deep, is significantly greater than the number of the other physiological groups of the saprophytic microorganisms (Table 2). The basic energy material for this physiological group of the soil microorganisms are the proteins from the soil organic layer. The nitrogen in the ammonia form is released as the product of their activity. In the winter all the processes of decomposition decelerated to a great extent, or

even completely stopped, owing to the low temperature. As a result, in the spring the soil organic layer is still rich in the protein nitrogen, preserved from the autumn period. It causes the considerable increase of the number of the amonifying microorganims in the soil organic layer and surface soil layer, in comparison with the total number of the microorganisms on the soil agar and the number of free nitrogen fixing.

Table 2. *Seasonal dynamics of the number of the basic physiological groups of the soil microorganisms in the soil under the common alder plantations (1,000 mo/1g zemlje)*

| Depth cm | Spring | Summer | Autumn | Average | σ | V |
|--|---------|---------|---------|---------|----------|-------|
| Amonifying microorganisms on MPA agar | | | | | | |
| Olth | 2,468.4 | 83.3 | 4,771.7 | 2,441.2 | 2,344.3 | 96.0 |
| 0-2 | 438.0 | 149.0 | 505.2 | 364.1 | 189.3 | 52.0 |
| 2-7 | 2,483.5 | 95.0 | 52.6 | 877.0 | 1,391.4 | 158.7 |
| The total number of the microorganisms on the soil agar | | | | | | |
| Olth | 647.4 | 8.3 | 13420.5 | 4,692.1 | 7,565.8 | 161.2 |
| 0-2 | 94.4 | 175.0 | 446.0 | 238.5 | 184.2 | 77.2 |
| 2-7 | 611.1 | 221.0 | 85.9 | 306.0 | 272.7 | 89.1 |
| Free nitrogen fixing on Erzbi's agar | | | | | | |
| Olth | 447.7 | 1,166.7 | 8,526.0 | 3,380.1 | 4,470.9 | 132.3 |
| 0-2 | 128.9 | 40.0 | 122.4 | 97.1 | 49.6 | 51.0 |
| 2-7 | 485.2 | 39.5 | 54.2 | 193.0 | 253.2 | 131.2 |

In the spring the total number of the microorganisms on the soil agar are the second-largest physiological group on the soil agar by the number. These microorganisms use the mineral forms of the plant assimilatives, mainly nitrogen, for the synthesis of their own cell substances.

The free nitrogen fixing are the third-largest physiological group by the number. These microorganisms use the nutrients of the wide C/N ratio as the energy material, and are satisfied with the small quantities of the nitrogen from the nutrient, which they decompose by the biochemical processes, and are able to use the atmospheric nitrogen as well.

In the summer in the soil organic layer the number of the amonifying microorganisms and total number of the microorganisms on the soil agar decreases to a great extent. It is partly the result of the low moisture of the soil and soil organic layer, and partly the result of the lower quantities of the protein nitrogen, which is decomposed as early as in the spring. The smaller number of the amonifying microorganisms, which use the proteins as the energy material, caused the reduction of the production of the mineral forms of nitrogen in the soil organic layer. As a result, the number of the microorganisms, which are formed on the soil agar and which use the mineral forms of nitrogen, decreased. In the summer the nutrient produced by the other, native species, and coarse wood fragments, is dominant in the soil organic layer. The vascular and

mechanical tissues containing the small quantities of nitrogen mainly originate from the common alder leaves.

Due to the change of the composition of the soil organic layer the number of the free nitrogen fixing significantly increases. Owing to the activity of the free nitrogen fixing, which use the atmospheric nitrogen for their own needs, the C/N ratio of the soil organic layer becomes narrower, since by dying of them the nutrients rich in this element are transported.

In the autumn the condition of soil moisture and soil organic layer is improved and at the same time the new litterfall, i.e. fresh energy material for the saprophytic microorganisms, is transported to the soil. The population size of all physiological groups extremely increases. A dominant physiological group is the total number of the microorganisms on the soil agar.

The total number of the microorganisms on the soil agar is the dominant physiological group in the soil organic layer under the common alder plantations in the average annual number of the soil microorganisms. These microorganisms induce the environment trophication and high quantities of the plant assimilatives which are available to the plants. The increase in the total number of the microorganisms on the soil agar in the comparison with the number of the ammonifying microorganisms point to the fact that in the horizon of the soil organic layer the decomposition processes is more dominant than the processes of the humus synthesis, as well as to the fact that the decomposition results in the end products, and that the plant assimilatives occur in the mineral forms.

Nitrogen is biologically accumulated in the humus materials in the soil, by creation of the first phase of mature humus, which occurs as a result of the mutual synthesis of amino acids and hynones (Kononova 1963). The amino acids are formed as the intermediate product of the decomposition of the proteins, and hynones (aromatic compounds) as the intermediate product of the decomposition of the organic compounds which do not contain nitrogen, as well as a result of the activity of the microorganisms. These elements are the nitrogen and carbon components of the humus. The humic acids are formed by the further polymerization of the stage prior to humus formation (Kononova 1963).

The narrow C/N ratio of the common alder litterfall causes the transformation of the greatest part of the carbon by the oxidation processes in CO₂, which is the end product of the decomposition of the nutrient. By the biochemical processes the insufficient quantities of the carbon components, which would bound with the nitrogen components in the initial stages of the humus formation, are formed. By contrast, there is a surplus of the nitrogen components which occur during the decomposition of the soil organic layer under the common alder monocultures. The amino acids, formed by the

processes of the degradation of proteins influenced by the proteolytic ferments (aminization) are subject to the further biodegradation.

By the influence of the ammonifying microorganisms and their ferments of deaminases and desamidases convert, by the activity of amines and amides, into NH_4 form (Savic, Jekic 1975). In the neutral and alkaline environments, as well as under the favourable oxidation conditions, the ammonia nitrogen is subject to the nitrification processes (Savic, Jekic 1975, Tesic, Todorovic 1988), i.e. under the influence of the nitrifiers by the activity of nitrites turn into the nitrates. Since the reaction of the soil solution to all three sample plots under the studied common alder cultures is neutral, and the conditions of the aeration of the soil solum are good (Miletic 2004), it can be expected that the significant part of the mineral nitrogen on the studied soil will occur in the nitrate form as well.

The rapid mineralisation of the organic nitrogen is not favourable for the ecosystem as a whole. The mineral forms of nitrogen are very mobile and are quickly lost from the soil. The ammonia nitrogen is subject to volatilisation (evaporation) from the neutral and alkaline environments, such as the studied deposits. Nitrates, the salts of the nitric acids, are very water-soluble and mobile. By the descending flows these elements are transported in the deeper parts of the soil solum, even out of reach of the root systems of the forest trees. Together with NO_3^- anion the carrying cations (Ca, Mg, K, NH_4 , etc.), which are also important elements of the nutrition of the forest trees, are transported to the deeper parts of the solum. According to Savic and Jekic (1975) nitric acid is the main chemical agent of the processes of the extraction in the neutral and alkaline soils. In the deeper parts of the solum, in the anoxidative environment, under the influence of anaerobic and facultatively anaerobic bacteria are reduced to the molecular nitrogen.

In the soil organic layer under the studied common alder monocultures in the spring the organic forms of nitrogen are intensively converted into in the mineral forms, which are available to the plants, since the ammonifying microorganisms is the dominant physiological group of the soil microorganisms, and this process is immediately followed by the microorganisms on the soil agar, which use the products of the decomposition of the ammonifying microorganisms. As early as in the summer the lack of the nitrogen is clearly visible, since the free nitrogen fixing is the dominant group in the soil organic layer.

The release of nitrogen by the common alder monocultures is not balanced over the year, since there is no high rate of the humus synthesis. Humus is the only stable and long-lasting source of nitrogen and other nutrients for the taller plants. The decomposition of humus decelerates, which also implies the harmonized release of the mineral forms of nitrogen over the year. As a result of the decelerated decomposition of the less water-soluble materials,

such as humus, there are no greater losses of the nitrogen or the other elements of the nutrition, as in the case of the rapid decomposition of the nutrient of the untypical nature to the end products of the decomposition.

4. CONCLUSION

The large quantity of the nutrients circulated in the artificially established common alder stands on the reclaimed mine soils of the Mining-Energy-Industrial Complex "Kolubara". The litterfall of the common alder contains the great quantities of the macroelements of the nutrition, particularly the large quantities of nitrogen. The great part of the soil organic layer decomposes to the end products under the influence of the saprophytic microorganisms, and only to a lesser extent it is transformed in the humus. The fast decomposition of the soil organic layer implies the conversion of the plant assimilatives from the organic forms into the forms which are available to the plants. Under the common alder monocultures the release of the mineral forms of nitrogen from the soil organic layer is most intensive in the spring, whereas it significantly decreases as early as in the summer. It is proved by the decrease of the number of the microorganisms which use the mineral forms of the nitrogen (ammonifying and mineralogical), as well as by the increase of the number of the microorganisms which use the atmospheric nitrogen (free nitrogen fixing).

In contrast to the common alder monocultures, which produce the litterfall of the narrow C/N ratio, the mixed cultures of it in combination with the species which produce the litterfall of the wider C/N ratio can provide the various energy material for the saprophytic microorganisms. The presence of the common alder litterfall in the soil organic layer under the mixed cultures can intensify the processes of the decomposition of the soil organic layer, which is produced by the other species, and which is of the wider C/N ratio. The decomposition of the other species, with the simultaneous decomposition of the common alder leaf litter, enables the formation of the sufficient quantities of the carbon and nitrogen components for the intensive processes of the synthesis of the mature humus.

Humus is the stable and long-lasting source of the nutrients for the forest trees. The formation of the greater quantities of humus under the mixed plantations will enable the more equalized release of the nutrients over the whole vegetation period. When applied in the mixed plantations on the deposols which do not contain the organic matter, which also implies that they are devoid of nitrogen, the common alder can significantly improve the nutrition of other tree species in the plantation.

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THE MICROORGANISMS POPULATION SIZE OF THE SOIL ORGANIC LAYER AND CHARACTERISTICS OF THE LITTERFALL OF THE COMMON ALDER (*ALNUS GLUTINOSA* GAERTN.) ON THE RECLAIMED MINE SOIL OF MINING-ENERGY-INDUSTRIAL COMPLEX "KOLUBARA"

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Summary

In the studied common alder cultures (*Alnus glutinosa* Gaertn.) it was reported that this species transports to the soil the litterfall rich in the nutrients which these species use in the nutrition. In the litterfall of the common alder the extremely high quantities of nitrogen were reported, which is the result of its' ability to use the atmospheric nitrogen by the symbiotic nitrogen fixation. Nitrogen is the basic missing elements of the nutrition on the reclaimed mine soils of the studied area. Along with nitrogen, the common alder transports the high quantities of other macroelements of

nutrition to the soil. The soil organic layer, formed under the common alder cultures, is subject to the fast decomposition under the influence of the saprophytic microorganisms. The soil organic layer decomposes to a great extent to the end products, and to a lesser extent is transformed into the humus. It implies the fast conversion of the nutrients from the organic forms into the forms which are available to the plants. As a result of the high quantities of nitrogen and other nutrients in the litterfall of the common alder, as well as because of the rapid decomposition of the soil organic layer formed by this litterfall, the common alder used in the mixed plantations can significantly improve the nutrition of other tree species, and enable the formation of the great quantities of humus, which is the more stable and long-lasting source of the nutrients for the plants.

BIOGENOST ORGANSKE PROSTIRKE I KARAKTERISTIKE LISNOG OPADA CRNE JOVE (*ALNUS GLUTINOSA* GAERTN.) NA DEPOSOLIMA REIK "KOLUBARA"

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Rezime

U ispitivanim monokulturama crne jove (*Alnus glutinosa* Gaertn.) konstatovano je da ova vrsta donosi na površinu zemljišta lisni opad bogat hranljivim materijama koje biljke koriste u ishrani. U opadu crne jove konstatovane su izuzetno velike količine azota, što je posledica njene sposobnosti da koristi atmosferski azot simbiotskom azotofiksacijom. Azot je osnovni nedostajući element ishrane na deposolima istraživanog područja. Pored azota crna jova donosi na površinu i visoke količine drugih makroelemenata ishrane. Organska prostirka koja se formira pod kulturama jove podleže brzom razlaganju pod uticajem saprofitnih mikroorganizama. Organska prostirka se većim delom razlaže do krajnjih produkata, a samo manjim delom transformiše u humus. To podrazumeva brzo prevođenje hranljivih materija iz organskih u biljkama pristupačne oblike. Zbog visokih količina azota i drugih hranljivih materija u lisnom opadu crne jove, kao i zbog veoma brzog razlaganja prostirke koju ovaj opad obrazuje, crna jova primenjena u mešovitim kulturama u značajnoj meri može da popravi ishranu drugih vrsta drveća i obezbedi stvaranje većih količina humusa koji predstavlja stabilniji i dugotrajniji izvor hranljivih materija za biljke.

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