

The Potential Effect of Biochar Application on Microbial Activities and Availability of Mineral Nitrogen in Arable Soil Stressed by Drought

Helena Dvořáková, Jakub Elbl, Irina Mikajlo, Antonín Kintl, Jaroslav Hynšt, Olga Urbánková, Jaroslav Záhora

Abstract—Application of biochar to arable soils represents a new approach to restore soil health and quality. Many studies reported the positive effect of biochar application on soil fertility and development of soil microbial community. Moreover biochar may affect the soil water retention, but this effect has not been sufficiently described yet. Therefore this study deals with the influence of biochar application on: microbial activities in soil, availability of mineral nitrogen in soil for microorganisms, mineral nitrogen retention and plant production. To demonstrate the effect of biochar addition on the above parameters, the pot experiment was realized. As a model crop, *Lactuca sativa* L. was used and cultivated from December 10th 2014 till March 22th 2015 in climate chamber in thoroughly homogenized arable soil with and without addition of biochar. Five variants of experiment (V1 – V5) with different regime of irrigation were prepared. Variants V1 – V2 were fertilized by mineral nitrogen, V3 – V4 by biochar and V5 was a control. The significant differences were found only in plant production and mineral nitrogen retention. The highest content of mineral nitrogen in soil was detected in V1 and V2, about 250 % in comparison with the other variants. The positive effect of biochar application on soil fertility, mineral nitrogen availability was not found. On the other hand results of plant production indicate the possible positive effect of biochar application on soil water retention.

Keywords—Arable soil, biochar, drought, mineral Nitrogen.

I. INTRODUCTION

WEATHER conditions changes in Central Europe are predicted for the future. Precipitation totals will be the same, but their layout will be changed in growing season. This situation will have a major impact on agricultural production and the natural ecosystems stability [10]. In the past decade, agricultural fresh water consumption has been rising due to not only water thirsty vegetables and meat, but also to the increase in biofuel crops [13].

Water represents very important part of soil, which is necessary for dissolution and transport of nutrients in soil (rhizosphere). Fluctuations in the soil water content leads to a significant change in the nutrients availability of soil not only for plants but also for the organisms. Key practices (bio-

technology) providing risk minimization of soil fertility reduction due to agricultural soil water loss including:

- Changes in crop rotations – increase of plants proportion with lower water requirements.
- New cultivation methods – improved soil cultivation to increase soil organic matter content.
- New methods of fertilization – the use of alternative fertilizers such as organic-waste compost or biochar.

Biochar is the product of thermal degradation of organic materials in the absence of air (pyrolysis), and is distinguished from charcoal by its use as a soil amendment [16]. There are many hypotheses as biochar acts on soil properties, according to [21]: Biochar can affect many physical and chemical properties in soil such as pH, water holding capacity, nutrient availability and soil structure. However, biochar is not a standard product but might be produced from very diverse source materials with different charring methods and thus the properties and effects can vary greatly between different kinds of biochar. Therefore in this study the biochar made only from deciduous tree have been used.

Another important substance that can increase resilience of the soil to the negative effects is compost. Many authors [7], [9], [18], [19], confirmed the importance of compost (organic residues/matter) for agriculture use. Compost application has a positive effect on basic soil properties: physical, chemical and biological fertility. Composition of the input substrate has a significant effect on the compost quality [7]. Compost amendment improves physical, chemical and biological soil properties, in particular by increasing available nutrients mainly in the organic soil fractions. The organic matter application to degraded soils considered to be a good environmental practice. Therefore, the compost application to the soil has become a common environmental practice for soil restoration, soil organic matter maintaining, degraded soils reclaiming and plant nutrients supplying [19].

The environmental impacts of biochar used in agriculture, including its effects on global climate change and soil ecosystem, have resulted in a growing interest in the fields of atmospheric science, geology, and environmental science in recent years [8]. Observed effects on soil fertility have been explained mainly by the pH increase in acidic soils or the improved nutrient retention through cations adsorption. Possible connections between biochar properties and soil biota along with their implications for soil processes have not been systematically described yet [16]. Soil may be regarded as an

H. Dvořáková, J. Elbl, I. Mikajlo, J. Hynšt, Olga Urbánková and Jaroslav Záhora are with the Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno 13, Czech Republic (phone: +420 545 133 324; e-mail: helena.dvorackova@mendelu.cz).

A. Kintl is with Department of Geology and Pedology, Faculty of Forestry and Wood technology, Mendel University in Brno, Zemědělská 1, 613 00 Brno 13, Czech Republic.

aqueous system in which soil moisture constitutes the physical linkage between soil, climate, vegetation and soil fauna. Reactions during the solid-liquid contact processes on the pore scale (dissolution, precipitation, sorption) affect water and nutrient uptake by plants, the global cycling of chemical elements and the soil formation in a long term. Soil functions are mainly based on nutrient flux, wettability, water and carbon storage that were affected by biochar application to a sandy-loam calcareous soil. In general, biochar influence on soil functioning is dependent on the type of biomass and the type of pyrolysis to produce the respective biochar [17].

In the presented paper effect biochar amendment on microbial activity and availability of nitrogen in soil stressed by drought has been investigated by laboratory experiment. The main hypothesis is that biochar addition affects microbes in soil along with their activity and thus the ability of soil to retain sufficient fertility level with the reduced availability of moisture.

II. MATERIALS AND METHODS

A. Experimental Design

The above hypothesis was tested by laboratory experiment carried out in the plastic experimental container according to [11]: Twenty-four PVC tubes were used as experimental containers and located in the growth box from December 10th 2014 till March 22th 2015. All containers with indicator plant *Lactuca sativa* (one plant per one experimental container) were kept in a growth box at 24 °C (daily temperature), 20 °C (night temperature) and 65 % humidity (for all 24h) with a day length of 12 h (light intensity 380 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) during the whole time of experiment. Each experimental container had the same proportions (height was 12 cm and diameter was 16 cm). These containers were filled with 1.5 kg of topsoil. Soil samplings was taken on October 25th 2014 in accordance with ČSN ISO 10 381-6 (ČSN - Czech Technical Standard) in the area of our interest – protection zone of underground water drinking source “Březová nad Svitavou” (in “Czech”). Local annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9 °C mean of annual air temperature. Topsoil samples have prepared (homogenized) separately; moreover soil samples have been sieved through a sieve (grid size of 10 mm).

To demonstrate the effect of biochar (B_{ch}) addition on individual soil properties in arable land stressed by drought, five variants of experiment (V1–V5; each one in three repetitions) with different regime of irrigation were prepared. The complete overview is shown in Table I. The water content in soil was maintained at 70 % of soil Water Holding Capacity (WHC) in variants V2, V4, V5 and at 40% in variants V1 and V3.

Variants V1 and V2 were fertilized with mineral nitrogen fertilizer DAM 390 (one hundred liters of DAM 390 contain 39 kg of nitrogen - 1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and 1/2 is in the form of urea). Variants V3 and V4 contained 45 g of B_{ch} per pot,

this dose of B_{ch} is in accordance with [16] representing 50 Mg/ha. Used mineral fertilizer DAM 390 is registered for agriculture use in the Czech Republic [10].

TABLE I
DISTRIBUTION OF THE LABORATORY EXPERIMENT

Variants	Irrigation regime	Characteristics
V1	40 % WHC	0.140 Mg N/ha
V2	70 % WHC	0.140 Mg N/ha
V3	40 % WHC	50 Mg B_{ch} /ha
V4	70 % WHC	50 Mg B_{ch} /ha
V5	70 % WHC	Control

B. Biochar Production

In this study, our team has used biochar made from deciduous tree in low temperatures using slow pyrolysis. Base material was pyrolysed at 350°C or 500°C.



Fig. 1 Deciduous tree biochar

C. Determination of Nitrogen Availability

Index of nitrogen availability was used for example by [11] and these authors suggest: Index of nitrogen availability is the method for measuring content of available nitrogen in soil. The whole method is divided into two procedures. The first procedure is used to determine the content of N_{min} before incubation. The second procedure is used to determine the content of ammoniacal nitrogen, which is released from the microbial biomass. Available soil nitrogen is estimated from NH_4^+-N . The method was discovered and described by [4].

D. Determination of Basal Respiration

Basal respiration (BAS) have been determined by measuring the CO_2 production from soils incubated in serum bottles for 24 h. Field moist soil (15 g) has weighed into each of three 120-ml serum bottles. Bottles have been sealed with butyl rubber stoppers and incubated at 25°C. After 3 and 24 h, a 0.5 ml sample of the internal atmosphere in each bottle has been analyzed by gas chromatography (Agilent Technologies 7890A GC System equipped with a thermal conductivity detector). Respiration has been calculated from the CO_2 increase during the 21 h incubation period (24–3 h). At the end of measurements, the total headspace volume of each replicate bottle has been determined by the volume of water required to fill the bottle. The measured amounts of CO_2 have been corrected for the gas solved in the liquid phase. The results are expressed per gram of dry soil and [23].

E. Statistical Analysis

The potential differences in microbial activity and nitrogen availability have been analyzed by ANOVA analysis with post-hoc Fischer LSD test. All data were analyzed in Statistica 10 software. Graphic processing of measured data was performed in Microsoft Excel 2010.

III. RESULTS AND DISCUSSION

The obtained results are divided into four sections: microbial activity, nitrogen availability for soil microbes, mineral nitrogen content in soil and finally plant biomass production.

A. Microbial Activity

Microbial communities in soil consist of a great diversity of species exploring their habitats by adjusting population abundance and activity rates to environmental factors. Soil microbial activities lead to the liberation of nutrients available for plants and are of crucial importance in biogeochemical cycling. Furthermore, microorganisms degrade pollutants and xenobiotics are important in soil structure stabilizing and organic matter conserving for sustainable agriculture and environmental quality [3].

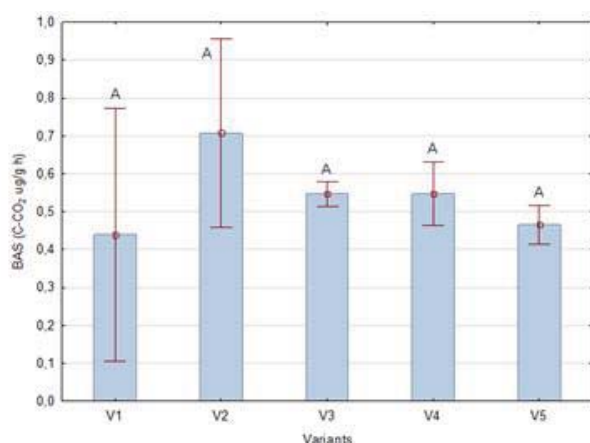


Fig. 2 Basal respiration (mean values $\pm$ standard error are shown; n = 4; different letters indicate significant differences in basal respiration at level $P < 0.05$)

Soil basal respiration represents one of the most important indicators of microbial activity in the soil. According to [1] the actual (basal respiration, BR) and the potential microbial respirometric activity (substrate-induced respiration, SIR) are well-established and widely used methods for microbial activity measurement in soil microbial ecology. The above results indicate that there are no positive effects of B_{ch} application on development of in soil. We did not found significant differences between microbial activities of individual variants. CO₂ released by the variant with B_{ch} addition has been the same or lower than the CO₂ production in variants with N addition or control. Similar results considering B_{ch} addition showed low ability in soil microbial

activities support has been published by [22]. Moreover the measured values do not indicate important influence of water content in soil on soil respiration. The optimal water content in soil for respiration is ought to be 50–70% of the soil water-holding capacity [5] and soil water regime have been maintained in the range from 40 % WHC to 70% WHC. Differences in WHC have been too small among individual variants. Therefore, significant drought effects on microbial activities have not been found.

B. Availability of Nitrogen for Soil Microbes

Ammonium N, which has been determined in filtered extracts after 7 days waterlogged incubation, indicates the amount of NH₄⁺-N in the microbial biomass. Nitrogen availability for soil microbes in arable soil has been determined according to [4].

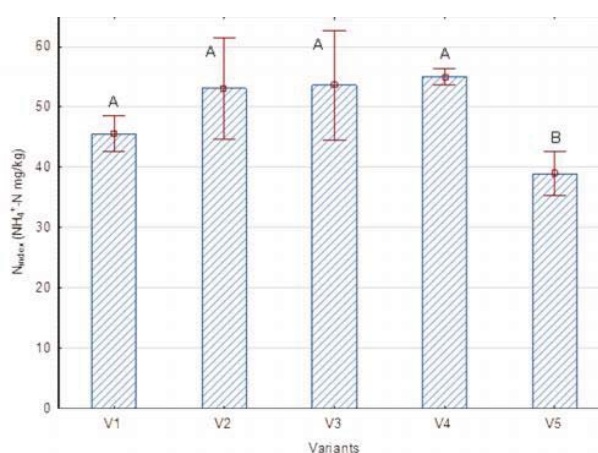


Fig. 3 Availability of NH₄⁺-N in microbial biomass (mean values $\pm$ standard error are shown; n = 4; different letters indicate significant differences in basal respiration at level $P < 0.05$)

Ammonium production during waterlogged incubation, i.e. index of nitrogen availability, is used regularly to determine the amount of nitrogen that has been stored in the microbial biomass [11]. Results which are presented in Fig. 3 show that there are no differences between N_{min} and B_{ch} addition on availability of NH₄⁺-N in microbial biomass. This situation may be explained by the fact that N_{min} and B_{ch} addition have not content organic nutrients which are necessary as a source of energy for soil microorganisms. Therefore, soil microbes could not use nitrogen from soil and store it into their bodies.

Influence of fertilization method (kind of fertilizers) and soil properties (soil water content, soil organic matter content, etc.) on quality and microbial communities' health in soil has been confirmed by [3], [5], [20]. Effect of fertilization method - nitrogen fertilization on availability of nitrogen in soil has been studied and reported by [6], [15].

C. Content of Mineral Nitrogen in Soil

Mineral nitrogen content (consisting of NH₄⁺-N and NO₃⁻-N) in the rhizosphere soil is an important indicator of threats to soil by nitrogen saturation [12].

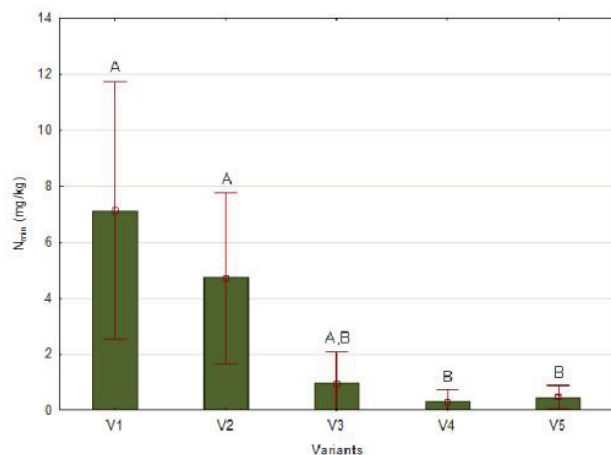


Fig. 4 The content of N_{min} in arable soil (mean values $\pm$ standard error are shown; $n = 4$; different letters indicate significant differences in basal respiration at level $P < 0.05$)

The B_{ch} addition effect on N_{min} content in arable soil is illustrated in Fig. 4. The above values indicate a negative effect of B_{ch} addition on nitrogen deposits in arable soil and thus on soil fertility (consider Fig. 5). On the other hand, these data are only partially significant. The highest N_{min} content ($V1 = 7.5$ mg/kg; $V2 = 5.4$ mg/kg) have been found in variants with N addition in comparison with variants without it ($V3$; $V4$ and $V5$). This situation can perhaps be explained by the fact that addition of N fertilizers ($V1$ and $V2$) leads to an increase in the contents of utilizable nutrients (N_{min}) in the soil.

Mineral fertilizers application increased the content of mineral nitrogen in the soil, but nitrogen has not been used by microorganisms (see Fig. 2; microbial activity stayed at the same level with no significant differences. On the other hand these nutrients have been used by plants (see Fig. 5; the highest plant biomass production has been found in variants with an applied N_{min} and water content has been maintained at the 70% of WHC). The relation between soil fertility and fertilization method along with soil water content has been reported by [2], [14]. These studies confirmed that N content in soil is dependent on the method of fertilization and fluctuations of soil moisture.

D. Plant Biomass Production

Lactuca sativa (salad) was used as indicator of effects of biochar and mineral nitrogen application on soil fertility.

Plant biomass production is illustrated in Fig. 5, this graph show significant differences only between the $V2$ and $V3$ and other variants. The highest production of plant biomass was found in $V2$ and conversely the lowest in $V3$. Mineral fertilizer was applied into $V2$ and soil water regime was maintained at 70 % of WHC. The optimal water regime and availability of N_{min} had a positive effect on soil fertility and thus on production of biomass. Conversely soil water regime was maintained at 40 % of WHC in $V3$. Moreover, only B_{ch} was applied there. B_{ch} does not (naturally) contain nutrients,

only carbon. Therefore application of B_{ch} did not affect soil fertility.

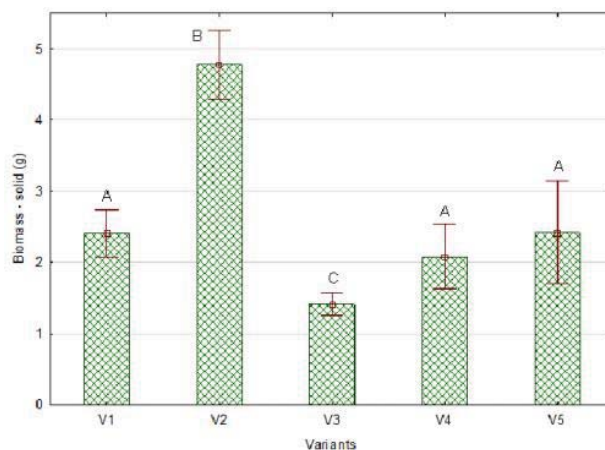


Fig. 5 Plant biomass production (mean values $\pm$ standard error are shown; $n = 4$; different letters indicate significant differences in basal respiration at level $P < 0.05$)

IV. CONCLUSIONS

The results presented in our study indicate that the application of B_{ch} can affect soil properties, but only with addition of other nutrients. Based on these results, we can conclude that the addition of B_{ch} can have positive effects on microbial activity and soil fertility, but these effects did not found. The authors are aware that the experiment was conducted under laboratory conditions and it should be repeated as a field-experiment.

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REFERENCES

- [1] N. D. Anayeva, E.A. Suysan, O.V. Chernova, S. Wirth, "Microbial respiration activities of soils from different climatic regions of European Russia", *European Journal of Soil Biology*, vol. 44, no. 2, pp. 147-157, 2008. C. Bimüller et al., "Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions", *Soil Biology and Biochemistry*, vol. 68, pp. 241-251, 2014.
- [2] J. Bloem, D. W. Hopkins and A. Bendetti, *Microbiological methods for assessing soil quality*. Cambridge: CABI Pub., 2006.
- [3] L. G. Bundy and J. J. Meisinger, *Nitrogen availability indices – Methods of soil analysis, part 2. Microbiological and Biochemical properties*, SSSA Book Series: Madison, pp. 951-984, 1994.
- [4] F. J. Cook and V. A. Orchard, "Relationships between soil respiration and soil moisture", *Soil Biology and Biochemistry*, vol. 40, pp. 1013-1018, 2008.
- [5] S. Delin and M. Stenberg, "Effect of nitrogen fertilization on nitrate leaching in relation to grain yield response on loamy sand in Sweden", *European Journal of Agronomy*, vol. 52, part B, pp. 291-296, 2014.

- [6] L. F. Diaz, M. de Bertoldi, W. Bidlingmaier and E. Stentiford, *Compost science and technology*, Boston: MA Elsevier, 2007, ch. 7.
- [7] Y. Ding, Y. Liu, W. Wu, D. Shi, M. Yang and Z. Zhong, "Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns", *Water Air Soil Pollut*, vol. 213, no. 1-4, pp. 47-55, 2010.
- [8] J. Elbl, J. Záhora, P. Škarpa, O. Urbánková and H. Dvořáčková, "The different effects of drought on soil microbial activities and soil hydrophobicity in permanent grass cover and arable land" *Ad Alta*, vol. 4, no. 2, pp. 57-61, 2014a.
- [9] J. Elbl, L. Plošek, A. Kintl, J. Záhora, S. Javoreková, I. Charousová, L. Kalhotka, and O. Urbánková, "Effects of drought on microbial activity in rhizosphere, soil hydrophobicity and leaching of mineral nitrogen from arable soil depending on method of fertilization," *World Academy of Science, Engineering and Technology*, vol. 8, no. 8, pp. 741-747, 2014b.
- [10] J. Elbl, L. Plošek, A. Kintl, J. Přichystalová, J. Záhora, J.K. Friedel, "The effect of increased doses of compost on leaching of mineral nitrogen from arable land", *Polish Journal of Environmental Studies*, vol. 23, no. 3, pp 697-703, 2014c.
- [11] J. Elbl, L. Plošek, J. Záhora, A. Kintl and M. Stroblová, "Effect of increased doses of compost to prepare reclamation substrate on soil respiration and content of mineral nitrogen in the soil", *Ad Alta*, vol. 3, no. 2, pp. 88-91, 2013.
- [12] K. R. Fingerman, G. Berndes, S. Orr, B.D. Richter and P. Vugteveen, "Impact assessment at the bioenergy-water nexus", *Biofuel Bioprod. Bior.*, vol. 5, pp. 375-386 (2011).
- [13] J. L. Gabriel, R. M. Carpena and M. Quemada, "The role of cover crops in irrigated systems: Water balance, nitrate leaching and soil mineral nitrogen accumulation", *Agriculture, Ecosystems & Environment*, vol. 155, pp. 50-61, 2012.
- [14] J. Galloway and E. Cowling, "Reactive nitrogen and the world: 200 years of change", *AMBIO: A Journal of the Human Environment*, vol. 31, no. 2, pp. 64-71, 2002.
- [15] J. Lehmann, M.C. Rillig, J. Thies, C.A. Masiello, W.C. Hockaday and D. Crowley, "Biochar effects on soil biota – A review", *Soil Biology & Biochemistry*, vol. 43, pp. 1812-1836, 2011.
- [16] G. Ojeda, S. Mattana, A. Avila, J. M. Alcariz, M. Volkmann and J. Bachmann, "Are soil-water functions affected by biochar application?", *Geoderma*, vol. 249-250, pp. 1-11, 2015.
- [17] L. Plošek, J. Elbl, A. Kintl, J. Záhora and J. Hynšt, "Influence of added compost on leakage of mineral nitrogen and biomass production", *Waste forum*, vol. 20, pp. 20-28, 2013.
- [18] L. Plošek, F. Nsanganwimana, B. Pourrut, J. Elbl, J. Hynšt, A. Kintl, D. Kubná and J. Záhora, "The effect of compost addition on chemical and nitrogen characteristics, respiration activity and biomass production in prepared reclamation substrates", *World Academy of Science, Engineering and Technology*, vol. 7, no. 11, pp. 483-488, 2013.
- [19] M. Sanaullah, E. Blagodatskaya, A. Chabbi, C. Rumpel and Y. Kuzyakov, "Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition", *Applied Soil Ecology*, vol. 48, no. 1, pp. 38-44, 2011.
- [20] S. Saario, K. Heimonen and R. Kettunen, "Biochar addition indirectly affects N₂O emissions via soil moisture and plant N uptake", *Soil Biology and Biochemistry*, vol. 58, pp. 99-106, 2013.
- [21] C. Steiner, W. G. Teixeira, J. Lehmann, and W. Zech, "Microbial response of charcoal amendments of highly weathered soils and Amazonian Dark Earths in Central Amazonia - preliminary results." In: Glaser, B., Woods, W.I. (Eds.), *Amazonian Dark Earths: Exploration in Space and Time*. Springer-Verlag, pp. 195-212, 2004.
- [22] M. Šimek, S. Virtanen, V. Křišťůfek, A. Simojoki and M. Yli-Halla, "Evidence of rich microbial communities in the subsoil of boreal acid sulphate soil conducive to greenhouse gas emissions", *Agriculture, Ecosystems & Environment*, vol. 140, no. 1-2, pp. 113-122, 2011.
- [23] B. Wolf and G. H. Snyder, *Sustainable soils*, Binghamton: Food Product Press, 2003.