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REVIEW OF *EX SITU* RESEARCH METHODS REGARDING THE PLANT - SOIL FAUNA RELATIONSHIP

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Abstract

While the plant community controls the quality and quantity of resources available to soil invertebrates, the soil invertebrates regulate plant growth and plant community composition. Soil invertebrates can modify plant traits, this effect cascading up to higher trophic levels, potentially thus determining changes in ecosystem functions. Thus, considering the special importance of this relationship between plants and soil invertebrates, our work aims to identify the various methods that support the study of this relationship. Although following the critical analysis of the literature, multiple methods were identified that highlight the interactions between underground and aboveground communities, we cannot claim that the study is exhaustive, which is caused by the immense number of works in the field. This aspect can only pave the way for new works and experiments to fill the knowledge gaps in this thematic area.

Key words: *ex situ methods, methods, plants, plants-soil fauna relationships, soil invertebrates.*

INTRODUCTION

The bidirectional relationship between invertebrate and plant groups is used as a bioindicator, providing information about soil quality, ecosystem services and ecosystem functioning (Scheu, 2002; Manu et al., 2019). Within different types of ecosystems there is a great diversity of functional traits of plants and soil invertebrates. Soil invertebrates play a key role in maintaining soil health and sustainability. Many species of invertebrates influence soil fertility and are very important in crop production and productivity (Manu and Onete, 2013). Soil fauna also is important in the regulation of nutrient cycling, in the decomposition process and also they can function as buffer organisms for various types of impacts (Carillo et al., 2011). The phylogenetic relationship between various invertebrate species is an indicator of abilities or constraints, on their morphology, physiology, and behavior. The question that arises is whether this relationship represents a sufficient condition for predicting ecosystem services and system behavior in the future (Walter and Ikonen, 1989). Ecological interactions occur between individuals and can change with life stage, season, time of day, physiological need, or in response to many other variables (changes within the food web, pollution, or other types of anthropogenic impact). The conceptual approach to plant-invertebrate interactions uses plant functional traits and soil food web characteristics (Cifuentes-Croquevielle et al., 2020). An important step in this approach is to include energy flow between species in the food web (e.g. building an energy flow network) (Scheu, 2002). Since plants use nutrients in inorganic form, they depend on the rate of mineralization in the soil (Araujo et al., 2004). Nutrient mineralization is mainly the result of the activity of soil fauna. The soil benefits from a great diversity of plant and invertebrate species, from a dense trophic network where the species living in it depend on its quality. Climate and land use changes cause modifications in all ecological systems including the structure and distribution of invertebrate communities (Wurst et al., 2018). Some studies mention that the soil cannot self-regulate its characteristics when links in the trophic network are impacted by rising temperatures, loss of moisture, pollution, etc. Due to the changes in the dynamics of the invertebrate community, there are also changes in the functional traits of the plants (Robinson et al., 2018). Soil fertility represents its ability to provide nutrients necessary for the growth and development of plants (Culliney, 2013) taking into account their ecological requirements (Boháč, 1990). A fertile soil can be defined either based on its properties or based on plant production and productivity (Benton Jones, 2012). Fertility is determined by physical factors (texture, structure, profile depth, water retention capacity, drainage capacity), chemical (pH, quantity of essential elements available to plants, ion exchange capacity, organic and mineral matter content) and biological (soil organisms, dominance-abundance ratio, inter- and intraspecific relationships) (Chiriac et al., 2020). Crop productivity and soil fertility can be affected due to the lack of essential elements. Sustainable soil management not only aims to improve crop productivity but also soil fertility and sustainability (Khalid et al., 2019). Studying all the species plus all their interactions is a daunting task, even in a simple ecosystem. In more complex systems it is impossible. Therefore, researchers need to reduce this complexity to a manageable size. In this sense, the aim of this literature review was to identify methods used by various authors to study these relationships outside their area of origin, in laboratory.

MATERIALS AND METHODS

The methods used to carry out this literature review included the following stages: public databases were queried (Web of Science, Google Scholar, Scopus), then the critical analysis of the specialized literature was carried out. All this information was compiled into a comprehensive Excel database containing many details from the literature reviewed. To carry out the query stage we used the keywords such as: microcosm, plant-invertebrate relationship, vegetation chamber, ex-situ methods for researching this relationship, etc. The articles used were exclusively free access or articles that were provided to us directly by the authors following the request made on certain platforms (ResearchGate). From all the articles studied, only those of them that contained the information targeted in this study were included in the present paper.

RESULTS AND DISCUSSIONS

Many ecological hypotheses cannot be proven with field studies, so experimentation in laboratory studies is used to provide insights into otherwise inaccessible interactions and mechanisms. The growing interest in recent years in the relationship between aboveground processes and soil ecology has been facilitated by the widespread use of laboratory experiments to overcome the limitations of understanding imposed by the particularly complex nature of the subsurface environment (A'Bear et al., 2014). Climate simulation chambers (growth chamber, vegetation chamber or phytotron) offer the possibility to test concepts in ecology and evolution, using different groups of organisms, including bacteria, algae, arthropods, etc. (Altermatt et al., 2015). These are artificial, simplified systems used to simulate the behavior of natural ecosystems under controlled conditions. They have long been used in ecology to increase scientific understanding of natural processes (A'Bear et al., 2014). In the context of changes in environmental conditions, the interactions of species on the surface of the soil and those in the subsurface represent a major concern in recent years. An important driver of these processes is the feedback between plants and soil invertebrate communities (Chiriac et al., 2020). Also, this type of experiment allows direct estimation of the effect of climate change on certain demographic traits (fertility, mortality, population growth rate, population density, etc.). These estimates can be used in population models to determine the extinction risk of a population in the absence of immigration or emigration (Cao et al., 2021). Experiments using a simulated climate chamber have the main advantage that they allow most variables to be held constant while only a few are manipulated, thus providing detailed insights into the ecology of soil plant-invertebrate interactions (A'Bear et al., 2014).

The mesocosm is defined in the literature as an experimental enclosure with a capacity of one liter to several thousand liters, for highlighting and clarifying the processes that occur during climate change (Stewart et al., 2013). Most subject areas in biology use certain models to explain phenomena. In the case of ecology, there are not a large number of models because everything depends on many different variables from year to year. The vegetation chamber is worth considering to create such models and make various predictions. Models need to have three useful characteristics: tractability, generality, and realism, which allow future experiments to build on past results (Srivastava et al., 2004). Greenhouse experiments were long ago considered irrelevant because it was believed that they could not be replicated and that there was no randomization between experimental designs (Lee and Rawlings, 1982). It is interesting, however, how a single term can have different meanings depending on the purpose and objectives of each study (Table 1). Also, different authors used a very different number of experimental variants. This is caused by the purpose and objectives of each individual work, the scale at which a certain experiment is carried out (Constantinescu et al., 2019), the variables included (amendment, inoculum, different types of soil) or the number of species of plants under study (a single species or a combination of several species). The number of pots/containers/pots of vegetation must be chosen very carefully, so that the requirements of performing some statistical methods are met, but also to capture the trends pursued by the study.

Table 1. Types of containers used in laboratory experiments by various authors over time

Year	**Authors**	**Containers**
1998	Fraser and Grime	35 outdoor, closed, ventilated plastic containers
1998	Salamanca et al.	plastic tubes in the growing chamber
1999	Scheu et al.	56 plastic tubes in the growing chamber
2000	Buckland and Grime	72 outdoor, closed, ventilated plastic containers
2001	Bonkowski et al.	48 plastic tubes
2003	De Deyn et al.	32 plastic containers in the growing chamber
2004	Cole et al.,	190 vegetation pots in the greenhouse
2009	Nygaard and Ejrnæs	72 outdoor, closed, ventilated plastic containers
2009	Aira and Piearce	30 vegetation pots outdoors
2010	Hedde et al.	glass jars with lids in greenhouses
2012	Borchard et al.	16 vegetation vessels in vegetation chambers
2012	Păun et al.	12 vegetation pots in vegetation chambers
2013a	Neagoe et al.	70 vegetation pots in the vegetation room
2013b	Neagoe et al.	48 vegetation pots in the vegetation room
2014	Nicoară et al.	10 vegetation pots in vegetation chambers
2015	Yang et al.	60 vegetation pots in vegetation chambers
2017	Panteleit et al.	100 plastic boxes with transparent lid
2019	Constantinescu et al.	5 vegetation pots in vegetation rooms
2020	Lebrun et al.	70 pots in the growing room
2021	Rubio-Ríos et al.	120 vegetation pots in vegetation chambers
2022	Balacco et al.	36 vegetation pots in the vegetation chamber

Vegetation chambers have been used since a decade and a half ago, being the most feasible for carrying out complex experiments accurately.

The authors of the analyzed studies used very different variables (temperatures, humidity and circadian cycles) (Table 2). This is due to the distinct purpose of each individual item. For example, Rubio-Rios et al. (2021) in their experiments used a temperature of 10°C because this was the average value of hourly records obtained during the same period of the experiment in previous years and a light/dark photoperiod of 12:12 h based on the length of the natural day cycle in that time of year. Usually, the variables to be set are based on knowledge of the ecological requirements (optimal values) of the species used in the experiment. One such study adapted the chosen day/night cycle in the vegetation chamber according to the species chosen in the experiment. Although initially the light period was set to 14 hours and the dark period to 10 hours (during the seeding period of the plant species chosen in the experiment), when the snail species was introduced into the experiment, the hours were changed as follows: 18 hours of light and 6 hours of darkness because this was more suitable for the growth of snails. In both periods, the temperature remained constant (23°C/17°C) (Scheifler et al., 2006). In other situations, the temperatures chosen are several degrees higher than the optimum of those species because the purpose of these studies is to make predictions of the behavior of various plant species in the current context of climate changes.

Table 2. Environmental variables used by various authors in greenhouse experiments over time

Year	**Authors**	**Temperature (degrees day/degrees night)**	**Humidity**	**Circadian cycle (hours day/hours night)**
1982	Lee et al	26°C/22°C		
1999	Scheu et al.	18°C/18°C		
2001	Bonkowski et al.	20°C/15°C		16 h/8 h
2003	De Deyn et al.	21°C/16°C		16 h/8 h
2004	Cole et al.			12 h/12h
2005	Bezemer et al.	20°C/14°C	60%	16 h/8 h
2006	Scheifler et al.	23°C/17°C		18 h/6h
2009	Aira and Piearce	20°C/20°C		
2010	Hedde et al.	10+/-1°C		11 h/13h
2013	Păun et al.	16°C/22°C	60%	
2013a	Neagoe et al.	22°C/16°C	60%	16 h/8 h
2013b	Neagoe et al.	22°C/16°C	70%	16 h/8 h
2014	Nicoară et al.	25-35°C /15-25°C		
2017	Panteleit et al.	27°C/27°C	90%	12 h/12 h
2017	Neagoe et al.	22°C/16°C	70%	16 h/8 h
2020	Lebrun et al.	24° C/21° C		16 h/8 h
2021	Rubio-Ríos et al.	10°C/ 10°C		12 h/12 h
2022	Balacco et al.	24°C/16°C	50%	12 h/12h

In the laboratory studies, the authors used plant species (Table 3) as indicators of certain types of changes (climate, land use, etc.) (Blouin et al., 2013). One of the studies manipulated plant functional diversity (monocultures and mixtures of low functional diversity and high functional diversity) in the presence and absence of detritivores and assessed the effects on litter decomposition, nutrient cycling and fungal and detritivore biomass. This study obtained positive effects of diversity on decomposition by detritivores. Among the species very often used in pot experiments introduced into the vegetation room is *Agrostis capillaris*. In one of the pots, the effects of microarthropod species diversity (Collembola) on nitrogen distribution between *Agrostis capillaris* and soil microbial biomass were tested to determine how the richness and diversity of soil fauna influences plant-microorganism competition for organic nitrogen (Cole et al., 2004). Another experiment using *Agrostis capillaris* alongside *Anthoxanthum odoratum* aimed to manipulate the composition of the belowground (no soil inoculation, invertebrate inoculation, microorganism inoculation or both invertebrate and microorganism inoculation) and aboveground (aphids and parasitoids) community composition to measure individual performance and population dynamics of introduced species. The authors were able to demonstrate that aboveground multitrophic interactions are influenced by the composition of belowground communities and thus, aboveground plant–insect links cannot be viewed independently of rhizosphere interactions (Bezemer et al., 2005).

Table 3. Plant species used in laboratory experiments by different authors

Species	**Nr. of articles**	**Article**
Achillea millefolium	1	Bezemer et al., 2005
Agrostis capillaris	6	De Deyn et al., 2003; Bezemer et al., 2005; Neagoe et al., 2013a; Wernitznig et al., 2013; Nicoară et al., 2014; Constantinescu et al., 2019
Anthoxanthum odoratum	2	De Deyn et al., 2003; Bezemer et al., 2005
Arrhenatherum elatius	1	Fraser și Grime, 1998
Campanula rotundifolia	2	De Deyn et al., 2003; Bezemer et al., 2005
Capsella bursa-pastoris	2	Johnson et al., 2011; Wagg et al., 2014
Cerastrium fontana	1	Bezemer et al., 2005
Deschampsia flexuosa	1	Wernitznig et al., 2013
Festuca ovina	3	Fraser și Grime, 1998; De Deyn et al., 2003; Bezemer et al., 2005
Festuca rubra	3	De Deyn et al., 2003; Wernitznig et al., 2013; Nicoară et al., 2014
Helianthus annuus	1	Păun et al., 2012
Hordeum vulgare	1	Johnson et al., 2010
Lactuca sativa	1	Scheifler et al., 2006
Lolium multiflorum	2	Borchard et al., 2012; Wagg et al., 2014
Lolium perenne	1	De Deyn et al., 2003; Balacco et al., 2022
Lotus corniculatus	2	Bezemer et al., 2005; Wagg et al., 2014
Lupinus angustifolius	2	Neagoe et al., 2005; Vișan et al., 2007
Lycopersicon esculentum	1	Yang et al., 2015
Nicotiana tabaccum	1	Neagoe et al., 2017
Oryza sativa	1	Panteleit et al., 2017
Phacelia tanacetifolia	1	Neagoe et al., 2013b
Plantago lanceolata	3	De Deyn et al., 2003; Bezemer et al., 2005; Wagg et al., 2014
Poa annua	3	Fraser și Grime, 1998; Scheu et al., 1999; Wagg et al., 2014
Poa trivialis	1	De Deyn et al., 2003
Prunella vulgaris	3	De Deyn et al., 2003; Bezemer et al., 2005; Wagg et al., 2014
Rumex acetocella	1	Bezemer et al., 2005
Rumex acetosa	1	Scherber et al., 2006
Rumex obtusifolius	1	De Deyn et al., 2003
Secale cereale	3	Neagoe et al., 2005; Vișan et al., 2007; Păun et al., 201
Senecio jacobaea	1	Bezemer et al., 2005
Senecio vulgaris	1	Johnson et al., 2010
Sinapis alba	1	Neagoe et al., 2013b
Stellaria media	1	De Deyn et al., 2003
Trifolium pratense	3	Scherber et al., 2006; Neagoe et al., 2013b; Wagg et al., 2014
Trifolium repens	1	Scheu et al., 1999
Tripleurospermum	1	Bezemer et al., 2005
Triticum aestivum	1	Bonkowski et al., 2001
Glycine max	1	Lee și Rawlings, 1982
Legumes, grasses, forbs	8	Vagg et al., 2014
Grasses and forbs	1	Buckland și Grime, 2000
grasses, forbs, and two woody species, *Echinacea purpurea*	1	Dybzinski et al., 2008
Deciduous, semi-deciduous, evergreen species *A. glutinosa* as key species	1	Rubio-Rios et al., 2021
grasses, legumes, small herbs, tall herbs	1	Partsch et al., 2006

Invertebrates were also widely used in laboratory experiments (Table 4). Earthworms, for example, have been recognized as ecosystem "engineers" and represent an excellent potential partner for humans in managing ecosystem services (Blouin et al., 2013). One such study investigated the effect of collembola and earthworms on *Poa annua* and *Trifolium repens* species.

Table 4. Invertebrate species used in the laboratory by different authors

Invertebrate species used in the laboratory	**Nr of articles**	**Article**
aphids	4	Fraser și Grime, 1998; Bezemer et al., 2005; Buckland and Grime, 2000; Johnson et al., 2010
annelids	1	Panteleit et al., 2017
mycorrhizal arbuscular fungi	4	Păun et al., 2012a, Neagoe et al., 2013 a, b; Neagoe et al., 2017; Balacco et al., 2022
arthropods	1	Panteleit et al., 2017
springtails	5	Scheu et al., 1999; Deyn et al. 2003; Cole et al. 2004; Partsch et al. 2006; A'Bear et al., 2014
diplopoda	1	A'Bear et al., 2014
earthworms	5	Scheu et al., 1999; Bonkowski et al., 2001; Partsch et al. 2006; Johnson et al. 2010; Hedde et al., 2010
herbivorous	1	Buckland and Grime, 2000
ground beetle	1	Buckland and Grime, 2000
isopoda	1	A'Bear et al., 2014
ladybird	2	Fraser și Grime, 1998; Buckland and Grime, 2000
trichopters	1	Rubio-Ríos et al., 2021
microorganisms	4	Bezemer et al., 2005; Păun et al., 2012b; Wernitznig et al., 2013; Nicoară et al., 2014
millipedes	1	Hedde et al., 2010
mites	1	Cole et al., 2004;
nematodes	3	Deyn et al., 2003; Bezemer et al., 2005; A'Bear et al., 2014
parasitoids	2	Bezemer et al., 2005; Johnson et al., 2010;
protozoa	1	Bonkowski et al., 2001
snails	2	Scheifler et al., 2006; Panteleit et al., 2017
oniscoides	1	Hedde et al., 2010

The authors started from the hypothesis that the soil used has a low amount of nitrogen available for plants and that the invertebrate species used could increase this amount. Earthworms caused a more than two-fold increase in the biomass of the plant species studied (Scheu et al., 1999). This is also confirmed by another study that demonstrated that decomposers (earthworms and collembola) influence soil structure and nutrient mineralization, as well as the activity and composition of the soil microbial community and therefore affect plant production and productivity (Partsch et al., 2006). The effect of earthworms on seed germination of *Lolium perenne* and *Agrostis capillaris* was also studied. The seeds of these species were added to vegetation pots at different depths, some with earthworms, others without, the experimental variants being improved with compost based on fungi (expanded clay). Germination was examined as a function of seeding depth and viability of seeds passed through the digestive tract of earthworms. Earthworms drastically reduced the germination of *Agrostis capillaris* seeds, but did not affect the germination of *Lolium perenne* seeds (Aira and Piearce, 2009). This was probably caused by the larger seed size of *Lolium perenne*. A similar experiment aimed to test the influence of earthworms and protozoa on phytophagous aphids on a host plant (*Triticum aestivum*). Both groups of animals significantly increased plant development, but the effects of protozoa exceeded those of earthworms at least twice, and aphids were more strongly influenced by protozoa than earthworms (protozoa caused increases in the number of juveniles and adults of aphids on each plant). The experimental variants also included microcosms with only protozoa or only earthworms, the ideal variant being when they were added together, being found to have a cumulative effect (Bonkowsky et al., 2001). Also in the laboratory, studies were carried out that used snail species. Both papers used the microcosm, but in the first article an evaluation of the transfer of heavy metals in the soil-plant-snail trophic chain was pursued, and the second aimed at quantifying the influence of detritivores (including snails) on straw decomposition of rice under flood conditions (Scheifler et al., 2006; Panteleit et al., 2017).

CONCLUSIONS

From a chronological point of view, I observed that the methods initially described were generally interested in the response of plants and/or invertebrates to certain changes in the environment. Currently, the studies are much more complex, they are multivariate, the researchers trying to find out the answer of the community chosen in the study in as complex a framework as possible. With the development of knowledge and the accumulation of experience of researchers, the studies were carried out at a more specialized level, scientists trying to conduct them in the laboratory. In recent years, the trend is towards studies carried out in vegetation chambers, where researchers could modify the environmental variables (temperature, humidity, circadian cycle) and the species used (plants, invertebrates, microorganisms) so that their results are as accurate in terms of forecasting the effects of global changes on the plant and invertebrate species studied. Over the past 30 years, more and more studies have appeared that aim to understand surface-subsurface interactions. The literature includes many papers that highlight the fact that these interactions have an essential role in the functioning of terrestrial ecosystems and in the provision of much-needed ecosystem services.

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