Evaluation of PhD thesis by Vojtěch Lanta: Biological diversity: measures of ecosystem functioning and implications for restoration ecology

The PhD thesis is based on seven papers, of which four papers are accepted for publication in international journals, two are submitted and one is at a manuscript stage. Vojtěch Lanta is the single author on one paper, and the first author on all other papers.

The thesis focuses on plant biodiversity in a very wide context with two main topics: 1) The effect of vascular plant biodiversity on ecosystem functioning defined as; plant productivity (paper I & II), weed invasions (paper III) and resistance in plant production (paper IV), 2) The examination of various restoration techniques as tools for re-establishing the native vegetation in former exploited peat land habitats (paper V, VI, VII).

Paper 1 examines the short-term effect (4 months) of species richness and functional group diversity on plant biomass at both high and low nutrient levels, and seeks to explore the relative importance of complementarity and sampling effect. This study was thus testing general biological theories related to the importance of biodiversity for ecosystem functioning. The results are interesting, and important for a further understanding of how biodiversity affects important ecosystem properties.

Paper II is a field based study that corresponds to the pot experiment in paper I, and thus gives a rare and very valuable possibility to compare the two different approaches for testing the effect of biodiversity on ecosystem functioning. However, one could argue that this advantage is not fully used (either in paper 1 or 2). As for paper 1, this experiment generate a large amount of results (perhaps an overload which always has a tendency to obscure the main messages), and my main concern is how these results are dealt with in the discussion which is not clear.

Paper III is based on the same experimental design as paper II, and examines the effect of biodiversity on weed invasions. Although this is a nice experimental design, the results are ambiguous, and the relationship between resident and weed species revealed in this paper do not correspond with the conclusions on the same issues (using the same data set) as in paper II; i.e. paper II concludes that "The biomass of weeds….were negatively affected by the diversity characteristics of sown species" (from the abstract), while you in some models in paper III found a positive relation between resident species number and weed invasions. In principal, all data referring to the resident and weed species relationships should be presented in the same paper (paper III).

Paper IV. This is a well designed experiment on the effect of slug herbivory on plant productivity in plant communities with different species richness. The results are interesting, but the discussion and partly also the introduction is unclear and in the need of a major revision.

Paper V. This is a well written paper on plant regenerations in formerly exploited peat lands. Although the study is descriptive and the analyses basically correlative, it's very informative and provides a useful complement to paper VI and partly also paper VII.

Paper VI. This seems to be an appropriate experimental restoration study were the objectives were to examine the plant ecological effects of manipulating the soil water level on the growth of *Betula pubescens* in both a greenhouse and a field experiment. However, the text is in a need of a major revision due to several errors in the text as well as unclear sentences which makes it difficult to grasp the main messages.

Paper VII. This is an appropriate experimental restoration study were the objectives were to examine the plant ecological effects of dam constructions in former drained mire vegetation in a mountainous region in the north of the Czech Republic. The experimental design and statistical analysis seems appropriate, but some of the results difficult to interpret. The discussion also had some unclear meanings. The writing is in a need of a revision.

Although this is a good thesis, as evaluated by the individual papers (and I will be more specific in my evaluation further down in this statement), it's not obvious to me why two such different topics were included in the same thesis. The first four papers (as I consider to be the best part of the thesis), would have been sufficient for a doctoral defence, and it would make it easier to write (and to read!) the general introduction and discussion for a more limited and well defined topic.

I am in general impressed by the experimental design adapted for six of the seven studies. The statistical analysis used seems appropriate, and these approaches together ensure the quality of the results which due to the experimental design are highly valuable. However, results from paper II and III are partly contradictive, and especially one paper (VII) suffers from an unclear presentation of results. Clarity in the result chapter is extremely important for the value of scientific writing, and I have made some major and several minor comments in the printed thesis (available after the defence). A similar weakness concerns especially the discussions in paper II, III, IV, VI, VII, and a major revision is needed to communicate the main messages derived from these important experimental results.

My conclusion is that the thesis by Vojtěch Lanta fulfils the requirements for a public examination.

Trondheim 27.04.2006

Gunnar Austrheim

Review of V. Lanta, 2006, Biological diversity: measures of ecosystem functioning and implications for restoration ecology. PhD Thesis.

The thesis includes 7 papers covering two broad topics: (1) the effect of the number of species and functional groups on biomass of vascular plants in artificial plant assemblages, including confounding effects of weeds and the effect of grazing by a generalist herbivore (papers I-IV), and (2) restoration of disturbed peat bog vegetation (papers V-VII). Four papers have been published in international and regional journals, two have been submitted and one is a manuscript. V. Lanta is the first author of all seven papers, indicating that his contribution was in all cases substantial.

The number of papers and amount of work done by V. Lanta, both in the field and in the lab, is admirable. The range of the studied topics and variety of methods presented in individual papers is also noteworthy. On the other hand, the unequal ambitions of individual papers, contrasting value of the obtained results, and, apparently also in the unequal effort put into individual studies, make the thesis unbalanced. While the diversity papers (I-IV) are generally based on sound hypotheses, well designed experiments, carefully applied statistical analyses, proper interpretation of the results and sound discussion, and the papers are written in a good English with a low number of spelling errors, the other set of papers (papers V-VII) sometimes suffer from a poor background, clumsy terms and spelling errors.

Following recommendations by Prof. Lepš, I separate more general and conceptual problems ("Main comments") with clearly formulated questions to which I would like to get answers in the course of the defence, and "minor comments" which can be eventually useful for the authors when revising the manuscripts. I do not list spelling errors because most of them can be easily found by available utilities.

MAIN COMMENTS

PAPERS I-IV

1. The idea that we can get new insights into functioning of ecosystems using a classification of organisms into functional groups is widespread. However, most authors utilise as surrogates of real functional traits the "old-fashioned" life-forms, plant size, leaf persistence, etc., i.e. ad-hoc selected easily obtainable traits. It is still rare to see a paper where authors search for functional traits according to their real and specific functional role. Consequently, significance of the results based on the post-hoc selected "functional" plant groups is not much different from that based on randomly assembled groups (Wright JP et al., 2006. Ecol. Lett. 9: 111-120). Can you imagine and describe how to classify plant species into groups according to the magnitude of the effects on ecosystem functioning? Which type of experiments will be needed to obtain relevant data? (see Wilson JB, 1989, Oecologia 80: 263-267 for a solution of a similar problem at a different organisational level).

2. Results presented in the thesis show that year-to-year changes in the complementarity and sampling effects were often great (significance, magnitude, even direction). Hence, an important part of the information needed to understand the precipitous dynamics of assemblages composed of fast-growing plants could not be obtained using annual frequency of data collecting. On the other hand, while more natural vegetation is not attractive for such experiments due to its slow response, weak effects and management difficulties. Do you see this as a problem? If yes, can you suggest how to approach or solve it?

3. For the greenhouse experiments relatively small pots were used (0.028 m^2). Final above-ground biomass was about 1–6 g. If these values refer to biomass per pot (as I suppose), then values of above-ground biomass ranged between 36 and 214 $g.m^2$. This corresponds to extremely unproductive grasslands or sparse stands in stressful conditions. Root-to-shoot ratio was low, especially in mixtures containing 8 and 16 species (about 1:5). This indicates that plants heavily suffered from low photon flux density. No information is provided about spatial arrangement of the pots in the greenhouse, e.g. distance between neighbouring pots. Still, I suggest that in such stressful conditions, identity of plants in neighbouring pots is very important. It does

matter, whether a target plant has as its neighbour a monoculture of a small plant (about 1 g of above-ground biomass) or a vigorous stand of 16 species (up to 5 g). Further, it is not clear how side effects (in pots situated along the sides of blocks of pots) were treated. I also did not find any information about germination percentage, germination sequence of individual species and establishment of plants in the pots. Number of sown species is not the factor used in the analysis. You always used species density. This may differ from the number of sown species, but no information is provided in any of the presented papers. Did all sown species germinate in all pots, established there and survived until harvest so that "Number of species and functional groups" used in analyses did not change in the course of the experiment? Were they abundant enough to contribute substantially to the "ecosystem function"? Could much of the noise in the data be caused by these factors? (I find the level of noise considerable in papers I-IV; note that it may mask effects you were interested in). In a short-term experiment, like that one presented in paper I, plant phenology belongs to important characteristics likely affecting the results. This experiment was finished within 4 months. Considering life cycle of the 16 species used in the experiment (and even the length of a vegetation season), the duration of the experiment was very short. Could the results of the experiment be different if it was prolonged to 5 months? or shortened to 3 months?

To ask the question: It concerns the potential confounding effect associated with the design of the experiment. In particular, I would like to learn more about the (potential) magnitude of the following effects:

neighbour effects
side effects
germination percentage
germination sequence of individual species
establishment, survival
difference between the number of sown and growing species
phenology and duration of the experiment

4. I wonder how to explain the contradiction between the idea that niches of plants differ more in the below-ground than in the above-ground and the apparent morphological and ecophysiological uniformity of roots in comparison with shoots. The explanation based on more heterogeneous soil in comparison with the room above soil surface is not convincing. How does the idea fit to the difference in symmetry of competition in above- and below-ground?

5. The methods of separation of below-ground biomass into species even from pots where 16 species is not described. Have you estimated the expected error of the procedure? I wonder how reliable these results are.

6. The fact that Legumes obtain nitrogen through N-fixing bacteria does not imply that they do not acquire it also from the soil, similarly to other plants. In the first months of their life is releasing rate of nitrogen in the form of root exudates negligible so that they cannot supply other plants by nitrogen. Discussion on p. 28 is therefore flawed.

PAPER 3

1. Invasion is relatively well established term (Richardson et al. 2000) with a clear meaning, different with that adopted in this paper. Weeds established in your experiment from a local seed source whereas the seeds of the sown plants were brought from outside. Consequently, the sown plants fit to the usual definition of invasive plants even better than the weeds. Hence, the Discussion, based on a comparison with real invasions (resistance to invasion, etc.) is misleading. Could you suggest a better term for what you have called invasion?

PAPER 4

1. I wonder what was the origin of the extreme complementarity values in grazed plots with 4 species.

2. To minimise variation in slug consumption, the slugs are usually kept starving for some time before they are used in the experiment. Was there any reason to skip this step in the experiment?

3. I suggest an alternative explanation of the results: Plant consumption by slugs decreased in mixtures of plants due to a lower accessibility of the food (too much time and energy spent by searching for it). It does not matter whether the obstacles were formed by plants with a low palatability or by twigs and stones. If this was the case, it has little sense to relate resistance to invasion and to the number of plant species (p. 85). Would you like to comment this?

PAPER 6

1. This paper presents completely opposite results obtained in the field and in a greenhouse. This discrepancy is explained by a contrasting water regime. I do not understand what can we learn from combining the two data sets. In my view, the water regime in the greenhouse experiment should be modified to be useful for explaination of the field data. Explain, please.

MINOR COMMENTS

PAPER 2

1. Spatial design of the experiment is not described. Were individual plots separated by any strips of the land? Even size of the experimental area is unknown. No evidence is provided to support the idea that the entire experimental area was homogeneous.

2. Data from individual years were treated as independent in GLM analyses, which is not substantiated.

3. The enormous variation in biomass values was likely caused by collecting samples from only two rectangles. Much more accurate estimates would be obtained if more smaller samples were pooled (see de Vries, Matveeva, etc.).

4. How would you explain the weak effect of fertilisation on above-ground biomass? Did you check what was the fate of nutrients added by fertilisation?

5. Why has one x-axis in Fig. 1 labels different from all other cases?

6. I failed to find the explanation of the term "biodiversity" in Tables 2 and 5.

7. The idea that Legumes do not compete for nitrogen with other plants is wrong.

PAPER 3

1. Table 3: data from individual years are not independent. They should be treated accordingly.

2. The potential role of the seed bank seems to be underestimated, the possibility of local extinction and repeated establishment either from seed bank or from outside should be considered.

3. Why are negative values of **r** presented as "-r = 0.13" instead of the usual "r = -0.13"?

PAPER 4

1. I miss units of above-ground biomass. If SD error bars are shown on a log scale, they cannot appear symmetrical (Fig. 1).

2. The chapter "Experimental design" should be re-organised.

3. It is not clear whether any of the sown species died during the experiment or was completely eaten by slugs.

4. p. 85 As fitness was not studied, it makes little sense to discuss it.

5. Was the electivity index N-distributed, to fulfil requirements of ANOVA?

6. How do you explain the fact the low values of the selection effect obtained for 4 species?

7. Fig. 2, the text (last paragraph on p. 82) and Table II are in conflict.

PAPERS 5-7

1. The English is getting worse: spelling errors, clumsy terms and phrases.

PAPER 5

1. The cited book by Moravec et al. did not convince me that it was necessary to combine plots of different sizes.

2. UPGMA and Ward's methods are two different approaches of clustering, not a single method. Which one was used?

3. Interpretation of the data as successional stages is complicated by the different time of onset of the succession in individual parts of the study area.

4. p. 98: "... tend to decrease ...", the same situation in Fig. 6 is correctly described as "no correlation"

5. Results of environmental analyses are interesting but unfortunately, they did not stimulate closer look at the problems.

PAPER 7

1. Selection of the "functional groups" can hardly be less functional: mosses, forest and mire species.

2. The non-significant time-position interaction does not imply that diversity was not affected by dam construction "numbers of species were higher ... above the dam".

CONCLUSION

Even if the above list of comments, questions and remark is quite long and by far not exhaustive, the thesis by V. Lanta convinced me that the candidate is a competent plant ecologists, capable to produce sound scientific results. Therefore, it is my pleasure to recommend this thesis for the defence.

Klimeš

Leoš Klimeš
Třeboň, 28-4-06

Review of PhD thesis submitted by Vojtech Lanta

The study by Lanta is about the effects of biological diversity on ecosystem functioning and the implications for restoration ecology. He conducted this study in an experimental way, both at the fundamental and the applied level. In the fundamental part he investigated productivity-diversity relationships and the effect of herbivory. In the applied part he investigated which factors affected the establishment of species in disturbed peatlands.

The thesis concerns itself with an up-to-date subject. The experiments are set up in a scientifically sound way and evaluated properly (however: see further). The relevance of the research is acknowledged by the fact that four of the constituent chapters have been accepted or published by peer-reviewed international journals.

General points:
First of all I would like to say that I appreciate very much that the author has taken the trouble to write up his thesis in English and not in his national language, which would undoubtly have been more easy for him.

Secondly I like the combination of fundamental and applied research very much. Also the combination of field measurements with greenhouse experiments is a strong point.

Having said the above I must admit that I had sometimes trouble to understand the message. The thesis is not always concisely written, especially not for people who are not in depth familiar with multivariate statistics. Moreover, the author sometimes uses parameters or variables that have either not been defined or uses different names for the same variable. An example is Table 1 on page 115. I cannot find a definition of 'growth dynamics'. Is this RGR?

A second point of concern is the virtual absence of research of the underlying ecological mechanisms. All chapters are descriptive, admittedly at a high level with state-of-the-art statistical techniques. There is much speculation - especially in the discussions - about soil nutrients, competition for light and/or nutrients, seed bank composition and seed dispersal, the effect of gap size and similar topics without much actual research about these topics. I think the discussions are a bit too ambitious for the data.

Points to discuss

- The first point I want to stress is the problem of pseudoreplication in the field experiments (e.g. Ch. VI and VII). How has the candidate taken that into account?
- The second point is the relationship between productivity and species richness under high and low nutrient levels (Figure 2). The differences between the two treatments in belowground productivity in mixtures are rather small, except possibly for Holcus. All species produce worse in mixtures than in monocultures. Also the differences in above-ground

productivity are not exceptionally large, except for Holcus and three legumes. This all suggest to me the following: 1) There is P-deficiency, even in the high nutrient supply; 2) there is a shift in nutrient allocation from root to shoot when growing in mixtures, i.e. competition for light; 3) most species already grow at maximum rate at low nutrient levels. This all together suggest to me that the effect of species/functional group richness on productivity is minimal when compared to other factors.

- In combination with the previous point: you add high N levels, even at the low nutrient treatment.
- In chapter III the addition of fertilizer has no effect on biomass production from sown species, nor from weeds. How is this possible?
- In the chapters III you find some correlations between plant diversity and weed invasion that are not easy to understand in my opinion:
    - In Table 3 you find a positive relation between species richness and weed biomass.
    - In chapter 1 you find a positive correlation between species richness and total biomass
    - In Figure 1 (p. 65) you find a negative correlation between biomass of residents and biomass of invaders.

  How does this fit?
- Moreover, the negative relation between biomass of invaders and that of residents seems to increase over time, although the biomass itself does not increase but instead decreases. How can this happen?
- Again there seems evidence that there is Phosphorus limitation: e.g., Agropyron is stimulated by legumes. How does this fit in the picture?
- More general: what happened in your control plots with invader biomass over time?
- Chapter V. You find higher levels of P in sites where Eriophorum is growing and attribute that to some effect of this species. Could it be the other way around? In other words: Eriophorum grows there **because** there is more P? The same could be the case for other abiotic conditions such as water table fluctuations.
- Looking at figure 6 I conclude that to increase biodiversity I must:
    - Drain. This is contrary to my expectation
    - Have a large Eriophorum ring. I would expect that you have more competition for light then
    - Have much litter. I would expect that species have then problems with germination

  Please explain
- In Chapter VI I see
    - Better growth of saplings at the richer soil in the greenhouse
    - Idem at lower water levels
    - Better growth of saplings at higher water levels in the field
    - Biomass allocation to photosynthetic organs (leaves) under shaded conditions.
    - Large differences in water levels between greenhouse and field experiment

  To me this seems that there is an optimum in growth rate with respect to water level somewhere around –10 to –15 cm. I would also say that shading does **not** have a positive effect but instead is a stress factor for the plant. I expect allocation from roots to leaves. Actually we measure that in several non-woody species.

- Finally some discussions about bog restoration (Chapter VII). Your figures 3 and 4 suggest that there is no clear directional trend in vegetation succession over the years, but instead fluctuation. Moreover, the position below and above the dam differs between the years. This gives me the impression that –despite some rewetting- the system does not really develop in the right direction. Is that correct?
- Last but not least: what is the actual problem in this particular bog? You dig out drainage ditches and get a water table increase of about 10 cm (**in** the ditch). Your precipitation is 1400 mm/yr, your temperature 4 C, so I would estimate some 400 mm evapotranspiration, which leaves you with 1000 mm water surplus per year. Obviously all this water disappears. Is there maybe another hydrological problem?

Conclusion

Finally I want to emphasize that, notwithstanding my critical remarks, I rate this study certainly as having sufficient scientific value to be allowed to defend in a disputation for the Doctor Degree. Both the experimental set up and the attempt to combine base line vegetation science with applied work are strong points.

Groningen, 02 May 2006
Dr. Rudy van Diggelen