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Meetings of the Royal Botanical Society of The Netherlands

MEETINGS OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 25 FEBRUARY AND 28 OCTOBER 1994

A Membrane Cytoskeleton at the Plasma Membrane of Plant Cells

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Actin binding proteins (ABPs) in animal cells serve a variety of functions: maintaining cell shape and mechanical properties, regulation of vesicle–membrane interactions, coupling actin filaments to each other, vesicles and organelles, anchoring actin to integral membrane proteins, regulation of actin filament formation, transport along actin filaments and involvement in signal transduction pathways.

We began our studies by localizing spectrin-like proteins in plant cells. These were identified using commercially available antibodies directed towards animal spectrin (two directed against human red blood cells and one against chicken red blood cells). With all three antibodies, positive reactions were found with cells, tissues and membrane fractions of various origins such as potato protoplasts, suspension cells and developing somatic embryos of carrot, embryogenic callus of maize, root tips of maize and bean and inflorescences of cauliflower.

Native plant proteins were separated using isoelectric focusing or SDS-treated plant protein contained spectrin-like antigens. Such proteins were enriched in the plasma membranes as judged by Western blotting of cauliflower inflorescence membranes separated using SDS-PAGE. This distribution is similar to what earlier has been found with actin (Sonesson & Widell 1993, *Protoplasma,* 177: 45–52).

Inside-out plasma membrane vesicles of cauliflower inflorescenses were immunogold labelled for transmission electron microscopy. Only a low percentage of vesicles was found labelled. However, *in situ* labelling of cauliflower inflorescences showed that only cells in the meristematic areas were labelled and that this labelling was not predominantly at the plasma membrane, explaining the low percentage of labelled vesicles with the immunogold technique.

We conclude that spectrin-like proteins are present, though not exclusively, at the cytoplasmic side of plant plasma membranes.

Cell Development and Cytoskeleton in *Micrasterias*

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Cell differentiation in *Micrasterias* is characterized by multipolar tip growth during which several areas of the cell grow at the same time, whereas the zones between cease their development. Early electron microscopic and experimental studies have revealed that the control mechanism for cell shape formation is located at the plasma membrane, which seems to carry zones of different properties for vesicle binding. High pressure freeze preparation for electron microscopy combined with freeze substitution has provided evidence that the number of fusions of secretory vesicles with the plasma membrane is increased at the latter zones. The areas where cell lobes are expected to form also correspond to sites of increased accumulation of membrane-associated calcium, which seem to arise from inward ionic currents at the growing tips. With regard to the cytoskeleton, microtubules, although present in the cortical cytoplasm are not involved in cell shape formation nor in the arrangement and distribution of cell wall microfibrils or cellulose synthase complexes at the plasma membrane. Since cytochalasin influences cytoplasmic streaming and cell growth and causes cell shape malformations it seems likely that actin microfilaments are involved in morphogenesis in *Micrasterias.* Recently, two systems of actin microfilaments have been visualized by microinjection of fluorescently labelled phalloidin and confocal scanning laser microscopy: a cortical actin network that covers both the growing and the non-growing semicell, and an actin filament aggregation around the moving nucleus which co-localizes with a microtubule system indicating an involvement of both cytoskeleton components in nuclear migration.

Leaf Anatomy of *Duguetia* (Annonaceae)

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The leaf anatomy of the genus *Duguetia* was surveyed with light microscopy, scanning and transmission electron microscopy. The genus is more or less homogeneous in its leaf anatomical features. The 27 species examined have dorsiventral or isobilateral leaves of 150–300 µm thickness and possess the genus-specific appressed stellate hairs and/or scales on the abaxial leaf surface. The more or less sclerified epidermal cells generally contain crystals (mainly druses). Oil cells are always present in both palisade and spongy parenchyma. Only a few species show deviating features. *D. neglecta* and *D. pycnastera* possess (tufts of) simple hairs, a very thin lamina and cuticle, and lack oil cells in the spongy parenchyma. *D. spec. nov.* and *D. uniflora* show upright stellate hairs of two dimensions and a very prominent midrib.

All leaf anatomical features studied are used for phenetic cluster analysis. Although the dissimilarities between most species were not very high, four clusters can be distinguished based on the discriminating features: the indumentum, the lamina and cuticle thickness, the thickness of the midrib and the distribution of oil cells in the mesophyll. The cluster containing *D. neglecta* and *D. pycnastera* is the most deviating cluster. The resulting phenogram is compared with the traditional classification of *Duguetia* into sections (Fries, R.E. 1934, *Acta Horti Berg.* **12:** 1–220; Fries, R.E. 1937, *Acta Horti Berg.* **12:** 221–288; Fries, R.E. 1939, *Acta Horti Berg.* **12:** 289–577). It appeared that the clusters based on leaf anatomy more or less agree with most sections (based on flower morphology mainly). From this analysis it can be concluded that most leaf anatomical characters cannot be used as systematic characters, but they should be included within the classification system of the genus. However, the different hair types found do possess some taxonomic significance.

The presented results have been published recently: Bakker, M.E. & W.J. Visser, 1994. Studies in Annonaceae XIX. Leaf anatomy of *Duguetia* St. Hil. (Annonaceae). *Bot. Jahrb. Syst.* **116:** 83–111.

Atomic Force Microscopy (AFM) of Botanical Specimens

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We present the results of an examination of various types of plant material with the Atomic Force Microscope (AFM). The AFM images of pollen grains were comparable to those made with the Scanning Electron Microscope. Sometimes convolution occurred but the images were reproducible and therefore are useful. Prior to examination, protoplasts of *Zea mays* were fixed in glutaraldehyde and dried on a slide. Imaging in liquid is possible with the AFM, but appeared difficult for protoplasts, as they hardly stick to the surface, such that the cell cannot wobble along with the scanning tip. New developments like the tapping mode, will improve this type of imaging. Most successful was the study of the texture of cellulose microfibrils of cell walls of radish root hairs. The cell walls were chemically extracted and the cellulose microfibrils were visualized with the AFM. The AFM showed a texture as known from the Transmission Electron Microscope. Pyrolysis-mass-spectrometry showed that the extracted cell walls were almost pure crystalline cellulose and so it is likely that the AFM images depict microfibrils free from hemicelluloses. A submicrofibrillar resolution could not be reached.

The Cytoskeleton of the Green Alga *Nitella pseudoflabellata*

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The internodal cells of the characean algae are cylinders (up to 100 mm × 1 mm) with conspicuous cytoplasmic streaming (up to 100 µm s^{-1}). It was formerly believed that there was a spatial separation of the tubulin and actin cytoskeletal components in these cells. Thus, microtubules were thought to be confined to regions near the plasma membrane where, in elongating cells, they are transverse and possibly associated with microfibril deposition. Furthermore, F-actin was thought to be primarily confined to subcortical regions where it is found in parallel bundles lying along chloroplast files, and where it generates cytoplasmic streaming by interacting with myosin-coated organelles.

However, recent advances in methodology such as the use of the actin-stabilizing agent MBS and the microinjection of FITC-conjugated phalloidin have revealed a more extensive actin cytoskeleton in the species *Nitella pseudoflabellata*. The actin cytoskeleton in elongating internodes of this alga is more reminiscent of the actin cytoskeleton in higher plant systems because of the numerous diverse and distinct actin structures that it contains, in addition to the subcortical bundles. These structures include actin rings around nuclei, actin rings around the chloroplasts immediately adjacent to the neutral line, and a reticulate actin cytoskeleton in the cell cortex.

Morphology of Pollen Tubes from *Pinus sylvestris*

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The ultrastructure, cytoskeletal organization and organelle movement was investigated in germinated pollen grains and pollen tubes of the gymnosperm *Pinus sylvestris* using the rapid freezing-freeze substitution technique, fluorescence and video microscopy. Morphological data on pollen tubes from *Pinus sylvestris* were compared to observations from a previous study on *Nicotiana tabacum*.

Pollen of *P. sylvestris* germinated and grew slowly compared to that of *N. tabacum*. A small percentage of the grains formed two tubes. After a few days, most tubes started to branch, giving rise to up to six side tubes. Main and side tubes showed a similar cytoplasmic organization and lacked the stringent apical zonation known from *N. tabacum* and other angiosperms. The grains and the distal part of *Pinus* pollen tubes were filled with large amyloplasts, and the cytoplasm was rich in lipid droplets and small vacuoles. Growing tips had a high density of secretory vesicles and tubular endoplasmic reticulum. Mitochondria were often positioned near the plasma membrane, in radial orientation, unlike in *N. tabacum* in which they are randomly oriented. Dictyosomes could be observed throughout the cytoplasm, often located between the mitochondria. In old tubes, large vacuoles with osmiophilic granules appeared near the tip. In *P. sylvestris*, as in *N. tabacum*, actin filaments stained with rhodamine-phalloidin showed a longitudinal orientation within grain and tube, with an intense staining in the tip, suggesting an accumulation of actin filaments in this region. In electron micrographs, microtubules appeared to be parallel to the tube axis, sometimes coaligned with microfilaments, but no microtubules could be revealed in the cortical area. Cross-sections of the wall showed a bow-like pattern of microfibrils, typical for helicoidal wall texture. The cytoplasmic streaming was generally slow: many organelles showed a brownian-like motion, but some others displayed a faster, directed movement, corresponding to a fountain-like pattern, thus reverse to that of *N. tabacum*.

From these results on the gymnosperm *P. sylvestris* it is concluded that the specific zonation of organelles in the tip of angiosperm pollen tubes, i.e. *N. tabacum*, is not essential for pollen tube tip growth.

Micromorphology of Neotropical *Begonia* seeds

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The genus *Begonia* includes about 1400 species, of which about 600 are known from the Neotropics. The neotropical species are arranged into 43 different sections. The validity and delimitation of a number of the sections is questionable. Seeds of about 240 neotropical *Begonia* species, representing 39 sections, were studied by scanning electron microscopy in order to obtain a better insight into the diversity of their seed structure, to explore adaptations to dispersal and to provide arguments for the sectional classification.

Although not as wide as in the African begonias, the neotropical species of *Begonia* show an appreciable diversity in seed size and structure. Mean seed length varies from 235 µm in *B. filipes* to 1450 µm in *B. fructicosa*. Seeds are mostly between 300 and 600 µm. In contrast to the African begonias (de Lange, A. & Bouman, F. 1991, *Wag. Agric. Univ. Papers* **91–4**: 1–82), the majority of the neotropical species have seeds of the 'ordinary type', which are dispersed anemoballistically. With the help of fruit-wings, the fruits are shaken by wind and seeds are released gradually by the pores and dispersed. This holds for larger sections, e.g. sections *Begonia, Gireoudia* and *Knesebeckia*, which mainly grow in more dry and open habitats and often have a wider geographical distribution. Their seeds show differences in micromorphological characters, such as the undulation of the anticlinal cell walls, type of anticlinal boundaries and cuticular ornamentation. A smooth cuticular surface is only seen in the monotypic section *Urniformia*.

A number of sections have a seed structure characteristic at the level of the section. All these sections have a more restricted geographical distribution and may deviate by growth form or habitat. Seeds may be adapted to anemochory by extended micropylar and/or chalazal ends with inflated, air-filled cells as in the Brazilean sections *Solananthera, Trendelenburgia* and *Enita* and in the Andean section *Rossmannia*, or by a more pronounced surface with deep, collapsed testal cells as in sections *Gobenia* and *Scheidweileria*. Only some sections have fruits and seeds not suited for wind dispersal. The epiphytic section *Trachelocarpus* has subsessile fruits hidden underneath the leaves, and seeds with a pronounced cuticular pattern consisting of stand-up or folding-over pleats. The dispersal agents are obscure and may be a combination of rain wash and of epizoochory on the legs, fur or feather of passing animals. The species of section *Casparya* from the wet Andean forest are rattleburrs, the fruits are provided with horns instead of wings. Their seeds have a very rough cuticle, often with a double structure, and may be secondarily dispersed by rain wash or epizoochory. No fruits, nor seeds with a distinct endozoochorous or myrmecochorous dispersal syndrome are known among the neotropical begonias.

Ornamentation of *Amorphophallus* pollen (Araceae)

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The first results of a morphological study of *Amorphophallus* pollen have previously been presented (*Acta Bot. Neerl.* **43:** 210). Since then the total number of species described has increased from 120 to approximately 160. Having studied a total number of 148 species with light and scanning electron microscopy, a fairly complete picture of *Amorphophallus* pollen is now available. Average pollen grain size is between 27 and 93 µm. Grain shape is spherical to ellipsoid, sometimes kidney-shaped. The grains are inaperturate; as yet, no polar and equatorial axes can be indicated. Examination of the pollen ultrastructure of 14 species with transmission electron microscopy demonstrated the presence of a distinct, spongy, acetolysis-resistant endexine and of a diversely structured ectexine without differentiation into foot layer, infratectal layer and tectum. The presence of a distinct endexine is remarkable. The exine in monocots is thought to be primarily ectexinous, and the endexine, when present, restricted to a weakly developed granular layer in the apertural areas. Usually, the ectexine in *Amorphophallus* pollen does not resist acetolysis. In many species it shows dark granules, which disappear during acetolysis. The osmiophilic nature of these granules suggests that an oily or fatty component is present, which might relate to the low resistance to acetolysis of the ectexine. Ornamentation is diverse: 13 main types have been distinguished, of which striate, psilate, fossulate, echinate and verrucate ornamentation are the most common. Various finely verrucate, areolate and scabrate types are less common. Comparison with an, as yet informal subdivision of *Amorphophallus* into 18 groups by W.L.A. Hetterscheid suggests that pollen ornamentation is of little systematic value. Striate ornamentation (64 spp.) is found in 13 groups, psilate (48 spp.) in 11 groups, and fossulate (14 spp.) in 9 groups. Adaptation to particular pollination types, well-known in Araceae, is a possible explanation.

MEETINGS OF THE SECTION FOR VEGETATION RESEARCH ON 19 APRIL AND 11 NOVEMBER 1994

The Significance of Ancient Phytosociological Data as a Reference

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Phytosociological data (relevés) are essential for demonstrating changes in vegetation, caused by both natural and anthropogenic influences. They are accurate documents of historical situations, and likewise can be used for monitoring developments. Particularly in the environment like the Dutch landscape, with a high level of eutrophication from nitrogen deposition and ground water enrichment, large-scale drainage, and changes in extent and pattern of traditional land use, the vegetation is highly dynamic. The applicability of phytosociological relevés arises directly from the high environmental indication value of plant communities. Compared to single species, the ecological amplitude of a plant community as a rule is much smaller; because of interspecific competition, the amplitude of the community is even smaller than the overlap in amplitudes of the composing species; this can be demonstrated by many examples.

The intended application of phytosociological relevés could be hampered (as far as more than local trends are considered) by an insufficient number of data. In The Netherlands, however, thousands of relevés are available due to a long and extensive phytosociological tradition, which—bearing in mind the small surface of the country—results in a rather dense network of data. In 1991, a long-term project was started to collect these historical relevés; the bulk of these are unpublished and hidden in grubby notebooks. Up to 24,000 relevés (1929–70) were brought together, of which 15,000 have been computerized meanwhile. The data are fairly well distributed over the country as well as over the various vegetation types. Endangered biotopes (like semi-natural dry grasslands, small-sedge fens and meadows, wet heathlands) are especially well represented.

Not only the floristic assemblage of each relevé but also additional information on location and time of inventory are computerized. On the basis of these data, temporal changes in distribution patterns of plant communities can be analysed. Examples are given of the *Cicendietum filiformis, Eleocharitetum multicaulis* and *Cirsio-Molinietum*. Especially in combination with floristic data, prognoses for successful restoration management and nature development in any area can be formulated, by compiling so-called predictive maps. Permanent plots are well-known tools to analyse concrete changes in vegetation (both fluctuation and succession). Of the numerous examples available, the vegetation development between 1933 and 1960 of a permanent plot near IJdoorn was chosen for illustration. After the closing of the Zuyderzee, a salt marsh community (*Armerion maritimae*) at this site changed into a swamp community dominated by tall forbs (*Filipendulion*). Ancient relevés also provide insight into syndynamic processes, due to both environmental (extrinsic) and synepiontological factors (intrinsic). The former is illustrated for the *Stratiotetum aloides* by comparison of the floristics of three relevés sets from different periods. Some plant communities in The Netherlands (such as the *Najadetum marinae* and the *Ranunculetum fluitantis*) are nowadays so much impoverished that historical data are absolutely necessary to understand their actual occurrence.

The Peener Haffmoor (BRD) as a Reference for Regeneration of Dutch Brook Valleys

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Information on the natural functioning of brook valley wetlands is needed to devise effective regeneration measures in The Netherlands. As it is impossible to find these types of wetlands in a relatively undisturbed form in The Netherlands, a study area in Mecklenburg-Vorpommern (Germany) was chosen. The peat stratigraphy of the nature reserve Peene Haffmoor shows that the original vegetation distribution is very similar to the situation in brook valleys in the Northern Netherlands, signifying a similar hydrological system governing this vegetation pattern.

Historical data show that the area has been used extensively for grazing, hay cutting and peat exploitation until the beginning of this century. The vegetation was disturbed, but has regrown after abandonment of the area, and the expected coarse zonation can still be found. A comparison of the present vegetation map with the original vegetation, reconstructed from peat borings, shows a similar vegetation distribution with local differences. Through hydrological investigations, coupled to a model of the regional hydrology these differences can be attributed to (i) influence of nearby groundwater extractions, diminishing the groundwater discharge on a large scale, (ii) a number of small polders adjacent to the area, locally disturbing the infiltration/discharge pattern, and (iii) the remains of old ditches, which conduct the eutrophic water of the river far into the reserve. The regional hydrological system appears to be largely intact.

It can be concluded that the Peene Haffmoor is a useful reference for the regeneration of Dutch brook valeys, as it provides a good example of fen development in a landscape where the hydrology is disturbed by human activities.

Reference Pictures from Abroad: Indispensable Standard for Nature Research and Management in The Netherlands

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All natural ecosystems in The Netherlands are more or less seriously affected by (i) surface reduction and fragmentation, (ii) disturbance of the abiotic base as a result of drainage, acidification and eutrophication, and (iii) lacking of system components such as large grazing and predatory animals.

Almost all existing ecosystems in The Netherlands are half-natural. Admittedly these are, as compared to natural systems (important in Dutch conservation programmes but actually hardly occurring in The Netherlands), small-scale and incomplete, but of great variation and beauty, and of considerable scientific importance. Most are now nature reserves. Nevertheless, the affects of drainage, acidification and eutrophication is predominant in all of them, resulting in serious degradation of these systems. On the plant community level this degradation manifests itself in (i) considerable loss of critical species, especially diagnostic species, (ii) increasing dominance of less critical, robust species, and (iii) increasing influx of species alien to the systems.

Intact reference systems are now very rare in The Netherlands. Such references are, however, essential standards for education, vegetation research, and nature management.

Since actual references are now almost non-existent in The Netherlands, there are two other possibilities: (i) historic Dutch vegetation relevés of intact plant communities, and (ii) actual examples of intact plant communities outside The Netherlands. Both kinds of reference are essential, but the importance of actual references from abroad is still underestimated. However, in order to be used as standards for ecosystems in The Netherlands, actual references from abroad must be chosen carefully. There must be sufficient similarity in climate, soil and hydrology, landscape and land-use, and species composition of the vegetation, resulting in plant associations of essentially the same character as those in The Netherlands. In general, these can be found in the Atlantic flora province of Europe. Therefore, the scope is restricted to parts of Ireland, England, Northern and Western France, Northern Germany and Northern Poland.

The half-natural grassland communities in particular are extremely degraded in The Netherlands, so attention is focused on this group, with examples of *Thero-Airion* (N. Poland), *Sedo-Cerastion* (N. Poland), *Nardo-Galion* (N. France), *Junco-Molinion* (N. Poland), *Calthion* (N. France, N. Poland), *Mesobromion* (N. France), *Arrhenatherion* (N. France), and *Agropyro-Rumicion* (N. Poland).

Reference Research on Natural Ecosystems in the West Siberian Lowland

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In Dutch nature conservation policy there is growing interest in the use of natural reference ecosystems. The national Nature Policy Plan (1990) is currently being worked out at a regional scale in regional plans for nature conservation and nature development. Especially when the *development* of large natural ecosystems is planned, good examples of similar, already existing natural ecosystems are important. It is increasingly difficult, however, to find such good examples in Europe. But a relatively good reference for that part of The Netherlands that is not influenced by the sea, i.e. the higher sandy grounds and the fluvial region, seems to be the vast and almost completely natural area in the province of Tomsk, part of the West Siberian Lowland. The region consists of undulating windborne sand deposits covered with mixed forests, mesotrophic fens and raised bogs, intersected by the river Ob and smaller rivers and brooks. The structure of the landscape resembles the original Dutch landscape closely. The climate is different of course. Nevertheless, there is a great similarity between the flora and fauna of this area and that of The Netherlands: 71–92% of the species of several taxonomic groups found in 1993 are indigenous in The Netherlands.

After two visits by people of the Utrecht University and the IKC-NBLF (in 1992 and 1993), a research project started in April 1994. This project will last until March 1995, but if the results are worthwhile it will be continued. In this project the Universities of Utrecht and Tomsk are working together. The main result will be an ecosystem typology (based on abiotic and biotic data) and a spatial system analysis, with special attention to hydrological processes.

How to Judge the Concept of 'Ecological References'

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Recently the term 'ecological reference' was introduced into Dutch 'nature policy'. The concept seems to play a central role in nature management and policy purposes, although its political and scientific meaning is not yet clear. The explicit use of ecological references stems from 1988, but the concept of references had already been introduced in environmental policy. Besides, conservationists used the 'well developed vegetation' as an implicit reference to judge actual situations. The concept 'ecological reference' adds some new elements, however. It is constructed out of historical and actual data, ecological principles and biogeographical information of species (F. Baerselman and F.W.M. Vera, 1989, *Natuurontwikkeling*, Min. LNV, The Hague). An ecological reference stands for an undisturbed, complete and sustainable natural system. The reference is a norm and, if possible, a goal for management.

The formulation of an ecological reference and ecological targets is useful if it is possible to predict the ecological processes and structures and if the targets are realistic in political terms. With respect to this, some lessons can be drawn from an analysis of the so-called Amoebe (Abstract Method for the Overall Examination of the Biological Ambience). In this approach on ecological reference is seen as a certain amount of individuals of a certain amount of species in a certain year (Min. V & W, 1989, *derde Nota Waterhuishouding*, The Hague). The goal is to restore the reference situation as much as possible. The analysis (De Bruin, J. *et al.*, 1992, *De Amoebe en onzekerheden*, University of Groningen) of the Amoebe shows several important difficulties related to the construction and use of an ecological reference. The study lists empirical problems (how to find historical data; what are the population dynamics of the species; what is the autecology of the species), theoretical problems (how to select the species; how to legitimize the sustainability claim; how to predict the amounts of individuals of a certain species) and political problems (what are the right measures to ensure the restoration; how can these measures by implemented). Although vascular plants and vegetation characteristics play a minor role in the Amoebe, probably most of the results of this analysis are valid for other kinds of ecological references.

A solution for these problems is to make a more realistic ecological reference. This means that only qualitative goals can be formulated. It is not advisable to formulate a vague ecological reference without concrete species because it makes a periodical evaluation almost impossible. An explicit indication of uncertainties can prevent the unrealistic expectations of policy makers. It is desirable to choose ecological targets for several time scales, dependent on the type of ecosystem and the intensity of man's use.

On the empirical level, collection of more data is valuable, but will not solve the problem of uncertainty. Data of ecosystems from the past or from elsewhere are only useful if these systems are comparable with actual ones. More insight is required in the dynamics of ecosystems, based on an evaluation of former predictions of natural processes. Besides, more attention has to be paid to theoretical problems with respect to the dynamics of ecosystems and the importance of determinism. Finally, especially for ecosystems in which human activities play a role, the societal context must be taken into account.

The most radical problem-solving strategy is to skip the whole idea of 'ecological reference' as an exclusive pathway. In practice, many bureaucrats seem to do so by choosing semi-natural and cultural references. This may be a realistic and less absolute position but it throws away a clear, stimulating and tempting idea. At least, empirical and theoretical studies can be started on the value of ecological references.

The State of Biodiversity Estimated in Terms of Occurrence and Composition of Vegetations

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Biodiversity is, according to the Nature Policy Plan, besides naturalness the most important quality indicator for Dutch nature.

In the report *Toestand van de natuur 2* (Bink, R.J. *et al.* 1994, IKC-NBLF, Wageningen) the state of nature in The Netherlands is described. In that report attention is paid to a selection of 24 plant communities which are relatively important for the conservation of biodiversity. They occur especially in the nutrient-poor parts of the higher sandy grounds and in the dune region. The occurrence of nearly all these plant communities has diminished in this century (in several cases even dramatically). Fourteen associations remained stable in the last decade. Examples of local regeneration give new hope for the future, but the problems caused by acidification, over-fertilization and desiccation remain serious.

In future reports on the state of Dutch nature the attention paid to the ecosystem level will shift from plant communities to so-called nature target types which are used in nature policy. Nature target types are descriptions of ecosystems in terms of target species from nine taxonomic groups (plants, invertebrates and vertebrates). Aggregations of plant communities form the basis of the description. The target species are used as quality indicators for each nature target type. They consist of endangered and internationally important species. In the near future the presence of target species will be measured in every existing ecotope in the nature reserves of the National Forest Service in order to determine the quality of these ecotopes (according to the description of the corresponding nature target types). It is important that this example will be followed in all the other nature areas that form together the National Ecological Network of The Netherlands.

Causes of Variation in Species Richness Between Plant Communities

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We explored which mechanisms cause variation in species richness between plant communities, because understanding these causes is important for various nature conservation issues. Furthermore, we investigated an existing dataset on plant species richness and various soil characteristics of 1570 fields of permanent grassland, collected in The Netherlands from 1930 to 1950 by Kruijne, de Vries and Mooi.

A first possible angle to the problem is to view species richness as the balance between colonization and extinction of species in a distinct area or community. The local colonization rate of species depends on the presence of a seed bank, on the vicinity of seed sources, on dispersal vectors (wind, water, animals, agricultural machinery) and on the presence of regeneration niches. The local extinction rate of species mainly depends on individual longevity, intraspecific resource competition, pathogens, physical–chemical stress factors (pH, anoxia, extreme temperatures, etc.) and mechanical damage.

A second approach is to explore the conditions required for coexistence of species, because coexistence of more species will result in a higher species richness. Coexistence is possible in so-called lottery systems or stochastic spatial models. Furthermore, differential resource limitation, resulting from a differential balance between uptake and losses, may result in niche differentiation which allows coexistence. Being developed along quite different lines, these modelling approaches recently appear to merge.

In our grassland data set, the highest species richness was found at low extractable levels of both phosphorus and potassium (no data on nitrogen availability). Whenever just one of these nutrients had a low availability, a much lower species richness was observed. This suggests that multiple nutrient limitation contributes to species coexistence. Furthermore, soil pH explained a large proportion of variation in species richness, with the highest species richness found at low P availability and high pH (>7). An interesting interaction was found between effects of pH and soil moisture. Under dry conditions the highest diversity was found at high pH (>7). Under wet conditions, the highest diversity was found in the weakly acid pH trajectory. These large pH and moisture effects are particularly difficult to understand from the existing plant species diversity models.

Some Mechanisms Behind the Increase of Species-richness During Restoration Management in Brook Valley Grasslands

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A fen meadow system which was not severely affected by drainage showed a good potential for restoration management, i.e. continued hay-making after the cessation of fertilizer application. The nature reserve in which the study was carried out features the gradual acquisition of grassland fields. This enables the study of both long-term succession in individual fields and the comparison of fields with different 'ages' of restoration management in a chronosequence. The latter is indispensable to unravel mechanisms behind the increase of species richness during restoration management.

Permanent plot studies over a 20 year period showed that restoration management resulted in a decrease of above-ground standing crop, an increase of species richness, and a replacement of species from eutrophic to mesotrophic soil conditions (Olff, H. and Bakker, J.P. (1991): *J. Appl. Ecol.* **28:** 1040–1052). An increase of species-richness was found in both individual permanent plots and in all plots per field, although in the latter case the species richness amounted to higher numbers. The chronosequence revealed a similar decrease in above-ground standing crop and an increase of species richness as the long-term permanent plots in an individual field. Hence, it was concluded that the chronosequence did represent the successional series.

Studies carried out in the same year in various stages of the chronosequence showed the importance of the fertilization history, i.e. the total amount of nutrients deposited in the field by fertilizer application and atmospheric deposition, taking into account the year when restoration management started, and the offtake of nutrients since then. The fertilization history affects the rates at which the former mesotrophic plant communities can be restored. It turned out that the cumulative nitrogen balance, i.e. the theoretical estimated balance between total accumulation since 1945 and offtake by restoration management, is a better predictor for the dynamics of the most important grass species *Holcus lanatus* and *Anthoxanthum odoratum* than just the number of years in which restoration management was carried out. This is also the case for the species richness per permanent plot, but not for the number of species in all plots per field (Bakker, J.P. and Olff, H., 1995: In: *Restoration of Temperate Wetlands* (in press). Wiley, Chichester).

When enough nutrients have been removed by offtake to decrease the cumulative nitrogen balance, other limiting factors for the increase of species richness can be found. Studies of the soil seed bank in various fields in the chronosequence revealed that the increase of species of mesotrophic soil conditions cannot be explained from the emergence from a long-term persistent seed bank reflecting the vegetation prior to fertilizer application, except for *Juncus acutiflorus*. All other species only occur in the seed bank if they are also found in the established vegetation, which makes it likely that they have a transient or short-term persistent seed bank (Bakker, J.P., 1989: *Nature Management by Grazing and Cutting*. Kluwer, Dordrecht).

Wild tunnel experiments showed that seed dispersal by wind is not likely to play an important part in the emergence of species during restoration management. Seeds of species in the established vegetation are proportionally attached to hay-making machinery during mowing. Seeds collected by machinery after cutting a late successional field can be released during mowing an early successional field. This suggests that most species which established during restoration management depend on dispersal by hay-making machinery.

Mechanisms of Species Diversity in Chalk Grassland

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In the recent past a number of models have been developed in vegetation science to predict general trends in species diversity. Various hypotheses have been tested experimentally, especially in grassland ecosystems. Fertilization treatments showed a negative correlation between productivity and species richness. However, in semi-natural grasslands repeated disturbances are also a necessary condition for maintenance of high species density.

In order to unravel the mechanisms behind species diversity in these grasslands, a long-term cooperative project was started in 1984 in both North America and Europe, applying the same series of experimental tests (Van der Maarel, E. & Sykes, M.T., 1993, *J. Veg. Sci.* **4**: 179–188; Willems, J.H., Peet, R.K. & Bik, L., 1993, *J. Veg. Sci.* **4**: 203–212).

Results of the experiments carried out in The Netherlands revealed that nitrogen (N) and phosphorus (P) are the most limiting elements in this ecosystem. The highest above-ground phytomass (*c.* 700 g m^{-2}) was measured in the plots treated with both N and P, compared to the control plots (*c.* 275 g m^{-2}). The number of species at the scale of 0·25 m^2 dropped in the NP plots by *c.* 35% within a 5-year period. The species number in the N plots (phytomass *c.* 400 g m^{-2}) decreased by 35%, too, whereas this number decreased with only 13% in the P plots (phytomass *c.* 600 g m^{-2}).

Light measurements (PAR) revealed that in both the NP and P plots the average light attenuation in the sward was highest of all treatments. However, small-scale light measurements showed a high degree in PAR reaching the soil surface in the P plots. Although the number of species which disappeared from the P plots in the course of time was as high as those in the NP and N plots, the newly established species in the P plots equalled those in the control plots. This implies that small-scale heterogeneity in vegetational structure is of decisive importance for regeneration possibilities, and hence for maintaining species richness in semi-natural grasslands.

Biodiversity of the Fauna in Relation to the Vegetation or Otherwise: The Concept of the Filtering of Species

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Diversity of animal species is often related to the plant species diversity of the vegetation. In some cases, however, other factors such as microclimate (vegetation structure), or human impact (frequency and intensity of management), have a dominant influence on faunal biodiversity. Diversity of soil microarthropods may be an example of the latter.

Decline in soil microarthropod biodiversity is described in a series, consisting of old forest stands, low-input grasslands, and high-input grasslands (with and without the use of persistent pesticides). Besides this decline, the patterns in the loss of species are analysed using classifications of tolerances to drought, of feeding guilds (Siepel, H. & de Ruiter-Dijkman, E.M., 1993, *Soil Biol. Biochem.* **25**: 1491–1497), and of life-history tactics (Siepel, H., 1994, *Biol. Fertil. Soils* **18**: 263–278).

Species which are intolerant to drought are absent in grasslands, but present in old forest stands. The main difference between low-input and high-input grasslands is found in the feeding guild structure of the community. Low-input grasslands are dominated by (herbo-)fungivorous grazers, whereas high-input grasslands are dominated by fungivorous browsers (with facultative phoresy). Fungivorous browsers (with thelytokous reproduction) are more abundant in forests than in low-input grasslands. Also, in other feeding guilds the fraction of thelytokously reproducing microarthropods is higher in old forest stands than in low-input grasslands. Application of DDT in high-input grasslands shows not only a high density of microarthropods with a high fraction of thelytokous reproduction, but also a decrease in the genetic variation of a thelytokously reproducing species. Patterns in species losses, the species filters, can be identified, using these methods. It is shown that the decline in biodiversity is not a random loss of species but that it takes place according to a predictable pattern.