Balance in the Biotope

Biodiversity provides many ecological advantages. Using large-scale field tests, **Gerd Gleixner** and **Ernst-Detlef Schulze**, scientists at the **Max Planck Institute for Biogeochemistry** in Jena, carry out research on biodiversity in meadows and forests, and explore its impacts on ecosystems and the Earth's carbon balance. Their studies also yield surprising insights into the factors that really serve the purpose of species protection.

TEXT **CATARINA PIETSCHMANN**

Grasses and flowers as far as the eye can see. The view is dotted with colored wooden pegs that demarcate the individual large and small plots. Thermal imaging cameras monitor a number of the plots from a bird's-eye perspective.

The Jena Experiment extends across an area of 16 hectares on the edge of the city and is bounded to the northeast by the Saale River. Behind it stand hills, some of them wooded and others speckled with meadow orchards. In the heat of the midday sun, there's not a single cloud in the sky – a perfect day for experiments with carbon dioxide labeled with the heavy carbon isotope ^{13}C. "Photosynthesis operates at full throttle only when it's really sunny, and the plants metabolize the labeled gas very quickly," explains Gerd Gleixner. He leads the Molecular Biogeochemistry Working Group at the Max Planck Institute for Biogeochemistry in Jena, and he wants to determine how the interaction between different species of flora and fauna affects the functioning of ecosystems, and whether greater diversity could buffer the effects of changing and extreme environmental conditions.

Along the back boundary of the meadow, around 20 students and scientists wearing straw hats as protection against the blazing sun crouch close to the ground on plastic stools. Metal frames stand on small squares of the meadow. On top of the frames are cubiform Plexiglas domes through which the labeled carbon dioxide flows onto the green test areas for 30 minutes. Following a specific timetable, the researchers cut grasses and plants, blade by blade, from the test squares and lay them on colored trays divided by species. These will be analyzed later in the lab to determine how quickly the special CO_2 was used to form sugars and to which specific parts of the plant they were transported.

Other students focus on the root area and puncture their plots like piec-

Fieldwork in the meadow: The Jena Experiment extends across a 16-hectare site on the edge of the city north of the Saale River. Scientists study how species diversity affects the functioning of ecosystems and the carbon balance of the soil there.

Photo: Catarina Pietschmann

Markus Lange (left) and Gerd Gleixner analyze the organic substances that are contained in the soil water and that reveal much about the processes at work in an ecosystem. The water collects in bottles in which a vacuum prevails. The containers stand in a tub that has been sunk into the ground.

es of Swiss cheese by removing soil and root samples. They sift the soil under canopies at the other end of the meadow. Like the cleaned and washed root systems, the soil is placed in sample bags for analysis.

The Jena Experiment has been running for 13 years now. When the 20-square-meter experimental area was established in 2002, it was just a field leased by Friedrich Schiller University Jena. The Max Planck Society purchased the seeds for planting using the organization's central funding, and the work began. Sixty species from four major plant groups that are typical of the Saaleaue meadows were planted: grasses, small herbs, tall herbs and legumes. They included plants as common as the dandelion, campanula, daisy, buttercup, red clover, alfalfa, scabiosa and ribwort. Each species was planted both as a monoculture and in combinations of 2, 4, 8, 16 and, finally, all 60 species. And the entire process was repeated four times in block strips parallel to the Saale River so that the distance from the river and the resulting differences in the soil could be compared statistically.

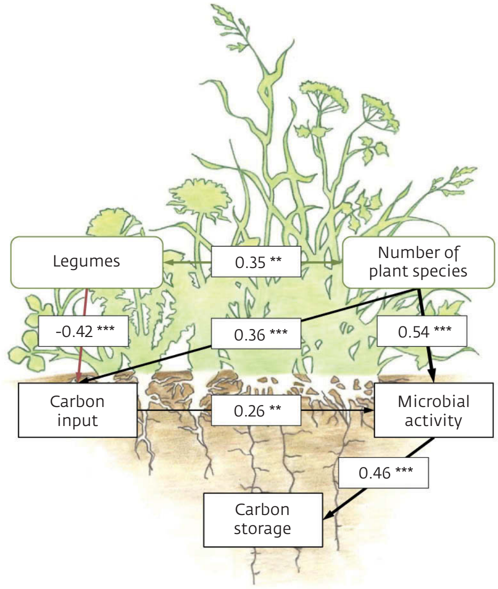
THE "WHO WITH WHOM" OF THE ECOSYSTEM

Only in some areas of the meadow, in the bare ground, was nothing at all allowed to grow, and this remains the case today. At the outset, even the earthworms were driven away from here with electric shocks. "If we want to study the carbon storage in the soil, we also need to know how it behaves when it has no vegetation at all," says Gleixner. The opposite extreme is represented by areas that were left to the forces of nature, on which whatever vegetation blows in or thrives is allowed to grow. These comparison plots are overgrown and are already starting to turn into forests.

To maintain the experiment, the parcels that were sown are cut and weeded twice a year by an army of students. The helpers remove every single plant that doesn't belong to the exclusive 60 species. The 480 test squares are otherwise left to their own devices (and those of the researchers). In addition to research groups from Friedrich Schiller University Jena and the Max Planck Society, scientists from numerous other German institutes, as well as Swiss and Dutch scientists from a DFG team of researchers, pursue their interests relating to the "meadow system" here. Botanists, entomologists, microbiologists, hydrologists and other experts use the area jointly to unravel the "who's who" and "who with whom" of the ecosystem – both above and below ground.

Gerd Gleixner is a biogeochemist and is primarily interested in the latter. His team's activities include taking soil water and soil gas samples, which trickle from the soil into bottles via narrow tubes. In the laboratory, the researchers extract the organic fraction, which comprises a conglomeration of numerous small molecules. "It's the finger-

Photo: Anna Schroll



How do the different characteristics of an ecosystem influence each other? What impact does this have on the volume of carbon stored in the soil? The black arrows indicate a positive influence, the red ones a negative influence. The closer the number on an arrow is to 1, the greater the influence. The more asterisks a number has, the more significant it is. A high number of plant species therefore increases the microbial activity in the soil and the carbon storage. The researchers therefore suspect that microorganisms in the soil result in carbon storage.

print of the ecosystem, so to speak," says Gleixner. Plants, microorganisms, worms and whatever else crawls through the soil leave living and posthumous traces in it, and Gleixner's team attempts to read these traces.

There's an entire hall at the institute filled with instruments for various kinds of chromatography, mass spectrometers and other high-tech equipment. The molecule cocktails are separated here in increasing detail and analyzed. Long-chain hydrocarbons, for example, usually come from foliage, and short-chain ones from microorganisms. "We're a long way from being able to identify every signal. However, we take all the information together, compare ecosystems and examine whether the same markers are found in the same systems," explains Gleixner. Members of his team have already travelled as far as Siberia, Tibet and Patagonia to compare ecosystems.

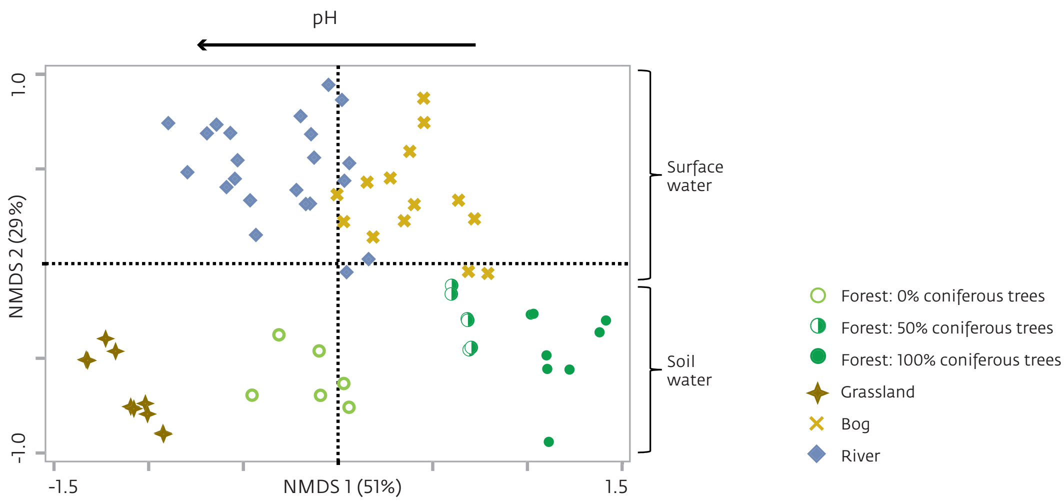
GREATER DIVERSITY MEANS MORE CARBON STORAGE

The researchers have long known that the biomass in a location increases with the diversity of the plants that grow in it – entirely without the help of fertilizers! The importance of this artificial supplement is clearly overestimated. "In agriculture, most fertilizer is spread in spring, gets washed away by the rain, and is of no benefit to the small plants," says Gleixner.

What's more astonishing, however, is that, with greater diversity, the volume of carbon and nitrogen stored by the soil also increases. Gleixner asks himself: Where does it all come from? What role do microorganisms play here and which ones are involved in this process? Do they work as separate groups, or is there a community effect? To find answers to these questions, he not only tests the chemical structure of the soil, he also analyzes the DNA and RNA of its minute inhabitants. Thus, species-rich meadows provide more nutrients to the soil organisms, increasing the productivity and genetic diversity of the microbial community. "Soil organisms are unselfish communists. They share everything – from food to their own genetic material. As a result, the population is constantly changing, and breaks down all carbon in the soil, in most cases to form carbon dioxide, or methane when there is little oxygen." >

Graphic: Nature Communications; photos: Anna Schroll (bottom left and right), Catarina Pietschmann (bottom center)

Gerd Gleixner (left) and his colleagues study, among other things, the volume of carbon stored in the soil. To do this, they take soil and plant samples to see exactly where and how much labeled CO_2 the plants have processed. The metal frame (center) was previously covered by a Plexiglas dome through which the marked carbon dioxide flowed onto the small plants.



Above: The molecular fingerprints of different ecosystems in the water: In a global project, thousands of organic substances in surface and soil water were tested using ultrahigh resolution mass spectrometry. This gives NMDS (non-metric multidimensional scaling) values, which act as ecosystem fingerprints.
The horizontal axis (NMDS 1) explains 51 percent of the differences in the data set and the vertical axis (NMDS 2) 29 percent. The substances extracted from water originating from coniferous, deciduous and mixed forests, as well as from grasslands, bogs and rivers, can be differentiated in this way. The pH value of the soil is an important factor here.

Below: Martin Göbel uses a ^{14}C accelerator mass spectrometer to analyze the content of the radioactive carbon isotope ^{14}C in soil samples. As the concentration of the isotope declines with the increasing age of the material, the tests provide information about the conversion and origin of the carbon in the soil.

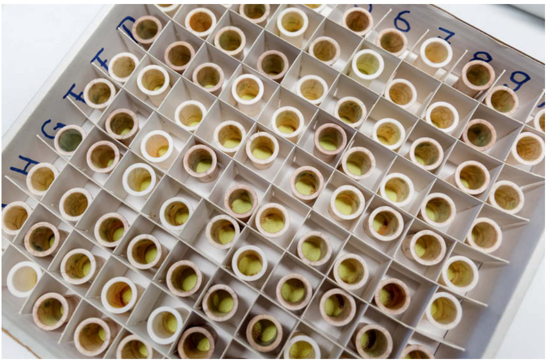
The microbes even process pollutants in this way, rendering them harmless. This is essential for clean drinking water. However, as demonstrated by the ^{13}C analyses of the green waste, part of the meadow community also benefits from this. "Ground-level plants like plantains, whose wide fleshy leaves practically lie on the ground, process primarily the CO_2 that diffuses from the soil and bind it again." For this reason, the researchers detected very little of the heavy carbon ^{13}C in them. "Tall grasses, in contrast, breathe in almost exclusively whatever is available in the air."

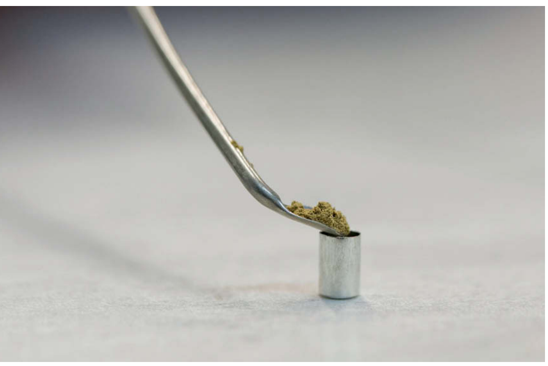
Whereas the researchers initially assumed that the material flows in a meadow are ideal if individual representatives of the four major plant groups are combined on it, they now realize that the presence of plants with different characteristics in an ecosystem is far more important – and the likelihood of attaining a thriving mix increases with the number of species. This correlation is tested in so-called trait-based experiments, in which plants are deliberately mixed based on two primary characteristics.

The first of these is the timing of their growth and flowering, and the second is the form of shoots and roots they develop. "Grasses grow fastest in the spring, while legumes still present strong growth in the fall," explains Gleixner. Over the course of the year, a good mixture of early and late species yields the best result. Multispectral cameras, which record individual test areas daily at different wavelengths, document the growth of individual plants and their levels of chlorophyll and other pigments. In terms of root formation, a good mix of shallow- and deep-rooting plants can make optimum use of the nutrients in the soil. What applies to the roots also applies to the leaves and flowers: high and low, wide and narrow – the more diverse the mix, the better the use made of the available resources, namely the sun, nutrients and moisture.

And multiculturalism makes ecosystems strong! "The resistance of the meadow to external stresses such as drought and infestations of pests and mice increases with greater diversity," says Ernst-Detlef Schulze, the initiator

Graphic: Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2014. Ecosystem-specific composition of dissolved organic matter. Vadose Zone Journal; photo: Sven Döring





Searching for traces in the soil: To measure the inorganic carbon and nitrogen content of the soil samples, the samples are weighed into and burned in small ceramic pots (top left) so that only inorganic substances remain. To trace the carbon and nitrogen metabolic pathways and rates in plants, the Jena-based scientists carry out experiments with heavy carbon and nitrogen isotopes. Franziska Günther analyzes the isotope pattern of biomarkers using a combination of gas chromatography and mass spectrometry (right). To carry out other analyses of the isotope ratios, the researchers weigh samples into tin capsules (bottom left) and then burn them.

of the Jena Experiment and guardian angel of the test meadow to the present day. "Our monocultures proved to be most sensitive. Most of them succumbed to harmful organisms." Even the otherwise indestructible daisy: Without spacers that prevent the direct transmission of infections from one plant to another, it contracts a fungal infection and dies. "It's the 'cold in a kindergarten' phenomenon," says Schulze, smiling.

Plant diversity also has a positive impact on the diversity of the other organisms – the animals. This was confirmed by a consortium of zoologists that focuses on aphids, grasshoppers, soil fungi, worms and plant pathogens. This was a new concept for nature conservation, which had previously been focused on the question of whether the butterflies should be protected or plant diversity. "The latter, obviously, because by doing so, we also protect the butterflies," says Schulze. Insects detect the smell of flowers from miles away and follow the scent like a red thread. Pathogens also find it easy to spread as their spores are carried over long distances by the wind. The soil organisms are slowest. Some of them haven't even managed to reach the middle of the test meadow yet.

"EVERY SPECIES HAS ITS MERIT"

The longer the Jena Experiment runs, the more interesting it becomes, says Schulze. Particularly where the plots weren't managed: one small meadow with each level of diversity hasn't been weeded since 2003. "We wanted to know how many species would assemble there." The answer is almost exactly 30. Not only do the ecosystems that started with fewer species level off at this number, but also those that started with significantly more species. "We constantly lose species in the plots where we originally sowed 60 species," says Schulze. "Assuming that 30 is the magic number for floodplains, it would be possible to calculate how many grasshoppers, butterflies, dragonflies, plant suckers and mice gather there."

So what is the most important insight gained from the Jena Experiment for Gerd Gleixner? "Basically, that every species has its merit. A large species pool ensures the survival of the plant community. And thus ours, too." Gleixner thinks in terms of very long periods of time, as demonstrated by his second research focus, the reconstruction of the paleoclimate. "If you look at the ice ages and how plant communities changed over major long-term climate developments, species that may be completely insignificant now could suddenly become important." For this reason alone, not a single one of them is extraneous.

"Basically, we have nothing to worry about," Gleixner adds, grinning. "We know that three million years after a major catastrophe, the plants are back. Whether the human race will still be around is a different question entirely."

But back to the present: For Ernst-Detlef Schulze, a research dream was re-

Photos: Sven Döring (3)

With the Biotree Experiment, Ernst-Detlef Schulze (right) and his team are seeking to establish how species diversity develops in different types of forests. The researchers also take climate data into account, which is recorded with, among other things, a weather station (left) in Kaltenborn, near Bad Salzungen in Thuringia.

alized with the Jena Experiment: repeating on the fertile loam soil of the Saaleaue alluvial plain the long-term experiment on the karstic sandy soil of Cedar Creek in Minnesota that yielded the very first insights into biodiversity. And because the situation around the turn of the millennium was propitious – the former GDR's old military and border areas were available for reforestation and amazing subsidies were available for such projects – Schulze went one better with the Biotree project. For Schulze, who is now 74 and still heads an Emeritus Group at the Max Planck Institute in Jena, this project was essential: "Herbs and grasses grow fast and easily, but one-third of our landscape is forest. The question as to whether the diversity laws we observe on the meadows also apply in forests was still completely open."

No sooner said than done: together with the Thuringia federal state forest authority, the researchers converted a military training area with sand and sandstone soil near Bad Salzungen, a tank training area with calcareous dry soil in the Thuringian Basin, and a fen with black soil on limestone into test areas. The bomb disposal units had barely packed and left when the researchers and their helpers got started with the planting of 5,000 trees on the 90 hectares of land. The test plots, on which monocultures, two, four or eight tree species grow in chessboard formations, are one hectare in size and divided into three parts. "One part was left to its own devices, the second was regularly thinned out, and the third was enhanced with rare tree species," says Schulze. For example, individual cherry trees were added to a mixed spruce-beech plantation.

MANAGED FOREST HAS GREATEST DIVERSITY

The early years were far from easy for the two-year tree nursery novices. "They had real difficulties asserting themselves against the grass and often had to be cut free and, in some cases, even replanted," explains Schulze. But in recompense for all their hard work, the researchers were rewarded with surprising findings on all plots. The tree species that prevailed were the most unexpected ones. For example, larches dominated on the extremely dry superficial and stony soils of the Thuringia Basin, while the beech trees failed miserably. "Previously, anyone wanting to grow deciduous forests had to plant at least one-third beech, or else they wouldn't be granted a subsidy. This was completely incorrect," says Schulze. Thanks to Biotree, the regulations have now been changed, and Thuringia forestry interns are now trained here on the test site.

Biotree met with great interest in Europe and was converted into an EU project for which comparable test areas were established in Belgium, Italy, Finland, Sweden and the Czech Republic. The experiment is now also part of the global TreeDivNet, which encompasses 18 similar projects comprising a total of almost one million trees in test locations ranging from Australia and China to Central America and Canada.

And what insights have emerged from Biotree, which is conceived as a 100-year-long endeavor? The greatest diversity is found in managed forests – and not in protected areas. "The most species-rich constellation is the even-aged stand in the deciduous forest," says Schulze. In even-aged forests, all of the trees are planted in the same year. The fact that the trees here were all planted in the same decade has no influence on the species diversity, but the fact that such forests are managed does.

Photo: MPI for Biogeochemistry (left), Thomas Stephan (right)

For this reason they have more herbs, more light indicators – namely species that don't tolerate a closed crown canopy – more deadwood, mosses, fungi and soil bacteria than any nature conservation area.

"As absurd as it may sound, the creation of a national park can reduce biodiversity considerably," says Schulze with a grin. "If a forest is placed under a nature conservation order, for example the Hainich forested hill chain in the Thuringia Basin, huge numbers of species are lost, particularly through game browsing." In contrast, not a single species has become extinct in a managed forest since records began over 250 years ago. Sustainable forest management thus offers the best species protection.

Ernst-Detlef Schulze has very clear views on the topic of nature conservation, and he can back them up with scientific evidence. "In Germany, 560 plant species are classified as endangered, and 42 have already become extinct. But 960 new species have emerged!" These are so-called apomictic species that don't reproduce sexually – that is, through pollination. In these plants, diploid cells – cells with two sets of chromosomes – assume the function of the fertilized ovum, so they reproduce asexually. These species are completely ignored by the Red Lists. "Whether we like it or not, we must finally acknowledge that a powerful evolutionary process is unfolding in our agricultural deserts!" Almost one-third of new species in Germany arose through malfunctions. "And this happened, not in spite of management, but because of it." Over 250 dandelion species formed vegetatively.

According to Schulze, nature conservation in Germany is based on three pillars that face in different directions. He places a Venn diagram on the table: there are 825 rare and potentially threatened species on the Red Lists for Germany. The number of legally protected species is 370. And then there are the 303 species that arise exclusively or almost exclusively in Germany and for which people are responsible. "But only 55 species are common to all three categories." This makes it practically impossible for forest and meadow owners to identify the plants they should tend with particular care. "There are endangered species that aren't protected and protected species that aren't endangered! Something's gone very wrong here!"

STUDIES ON THE BEST FENCING AGAINST GAME

Schulze really feels strongly about this topic. It's a good thing the forest itself is so peaceful. He himself has been managing a deciduous and coniferous forest in Thuringia for some time, and a small plot of "almost virgin forest" in Romania. His own deciduous forest, a delectable slice of diversity, as he describes it, contains 18 tree species, including old wild pear and apple trees. "Quite sour and inedible," he says.

In healthy ecosystems, the flora and fauna form a single entity, in meadows and in forests where wild animals hungry for leaves roam. Schulze carries out studies in his own forest on browsing damage and the best fencing systems for keeping game out. "In Romania I have bears, wolves and lynx, and here in Thuringia there are deer, stags and mouflon. And I have to regulate the stocks!" he adds. In full compliance with the shooting quotas, of course. This is also a controversial topic. But hunting and forestry share a particular task: to conserve the biological balance and species diversity in forests. Schulze has a hunting license of course and, like most hunters, he builds his own perches. This explains the plastic splint on his leg. He recently fell while building a perch and tore his Achilles tendon. Ouch! Ernst-Detlef Schulze waves dismissively, "Bah! It's not that serious." As far as he's concerned, species diversity and conservation are matters of far greater importance. ◂

TO THE POINT

- **In the Jena Experiment, scientists study how species diversity affects ecosystem functions on 480 meadow plots with varying levels of biodiversity.**
- **Test areas with higher levels of species diversity are more resistant to malfunctions, and their soil stores greater volumes of carbon.**
- **The number of species in floodplains that are left to their own devices levels off at around 30.**
- **The Biotree field experiment involving different forest ecosystems showed that the greatest species diversity is found in managed forests.**
- **The conservation of species requires a solution that unites the different approaches that currently exist and also takes the different locations of ecosystems into account.**

GLOSSARY

^{13}C-labeling: This is used, among others things, to trace the route taken by carbon in a metabolic process or chemical reaction. To this end, the heavy carbon isotope ^{13}C is added to a starting compound, for example CO_2, as a replacement for the usual ^{12}C carbon isotope. In various phases and following conversion, it is detected in the conversion products, for example in new biomass.

Diploid cells: These cells have a double set of chromosomes, while haploid cells have only one set. When an organism reproduces sexually, its gametes – eggs, sperm or pollen – are haploid.