Transcript from presentation by Professor Caroline Brophy, Trinity College Dublin, Ireland, summarising the paper: J. O'Malley *et al., Science*, 10.1126/science.ady0764 (2025)

SLIDE 1 – O'Malley *et al, Science*

- I'm going to share results from the LegacyNet project.
- LegacyNet is a large scale study on productive grasslands, which are grasslands used for example for forage or as a ley in a crop rotation.
- We were particularly interested in multispecies mixtures, by which we mean combining two grass, two legume and two herb forage species at sowing.
- In LegacyNet, the same experiment was conducted at 26 different sites across multiple continents.
- The first major results from LegacyNet are published in the leading journal Science.
- The key results from this paper are:
    - first of all, we showed that multispecies mixtures outperformed a single grass that had been treated with more than double the nitrogen fertiliser application;
    - secondly, we showed that multispecies mixtures outperformed a two-species grass-legume combination that had been managed at the same nitrogen fertiliser rate;
    - and we also showed that multispecies are a promising climate adaptation solution: we showed that the benefits of multispecies mixtures actually increased under warmer temperatures.

SLIDE 2 – Introducing presenting and paper title

- I'm Caroline Brophy, Professor in Statistics at Trinity College Dublin.
- And I'm delighted to share the details of our LegacyNet work that has been published in *Science*.
- The title of our paper is 'Multispecies grasslands produce more yield from lower nitrogen inputs across a climatic gradient'.
- This was a collaboration with many scientists with varying expertise from institutions across many different countries.

SLIDE 3 – The LegacyNet experiment

- The LegacyNet experiment was conducted at 26 sites and these sites spanned 15 countries across multiple continents.
- The same experiment was conducted at each site, where we manipulated species diversity of up to six forage species.
- The species used varied across the sites, but an example of a set of species is perennial ryegrass and timothy the grasses, red clover and white clover the legumes, and chicory and plantain the herbs.
- At each site, we had 47 plots that included monocultures of each of the six species, and mixtures ranging from two species up to six species.
- These plots were all managed at a moderate level of nitrogen fertiliser, which on average across the sites was 109 kilogrammes of nitrogen per hectare.
- We also had five additional reference plots where a single grass species was sown and they were managed at more than double the nitrogen fertiliser rate, so on average across sites this higher nitrogen rate was 261 kilogrammes of nitrogen per hectare.

- We measured the yield at each plot across all of these sites at multiple harvests that spanned multiple growing seasons.

SLIDE 4 – What did we want to find out?

- What did we want to find out?
- Essentially we wanted to know: can we improve the sustainability of productive grasslands while maintaining or improving agronomic performance?
- A traditionally used farming practice has been to sow a single grass species and manage it with high inputs of synthetic fertiliser, and while this is great for making the grass grow, there are environmental issues with using high inputs of synthetic nitrogen fertiliser. Right from the manufacture of synthetic fertiliser, and then once it has been applied to a grassland, you can have emissions straight away and you can have leaching into the groundwater.
- Previous research has shown the benefits of bringing legumes into a grassland, because of course legumes can fix atmospheric nitrogen in a natural process.
- An emerging practice in farming has been to sow a 70:30 grass-legume combination, which species wise, this is typically 70% perennial ryegrass and 30% white clover.
- But what about multispecies mixtures? How do they compare to these conventional practices?
- And if they have potential, in what proportions should you sow grasses, legumes and herbs?
- To address these questions, we modelled our data from across the LegacyNet experiment using the Diversity-Interactions modelling approach, and we specifically modelled the yield, which was measured in tonnes per hectare per growing season.

SLIDE 5 – Multispecies mixtures outperformed conventional practices

- On average across sites, we found that multispecies mixtures yielded 12.3 tonnes per hectare per growing season.
- While one grass species with high nitrogen fertiliser yielded 11.1 tonnes per hectare.
- So with multispecies mixtures, managed at a much lower nitrogen rate, we still found an 11% increase in yield.
- This is a key result for improving the sustainability of productive grasslands: we can get more yield with less nitrogen.
- You might ask if the same benefits can be achieved if just one grass and one legume species was sown, instead of a six-species multispecies mixture.
- Our predicted yield for one grass and one legume sown in a 70:30 ratio was 10.4 tonnes per hectare.
- So this means that going from one grass and one legume to a six-species multispecies mixture gave an 18% increase in yield.
- So for the same input of nitrogen fertiliser, we can do better with a six-species multispecies mixture compared to a 70% *Lolium perenne* and 30% *Trifolium repens* combination.

SLIDE 7 – Predicted yield

- We have conclusively shown that you can do better with a six-species multispecies mixture compared to one grass at high nitrogen, and compared to a 70:30 one grass one legume combination.
- But in what proportions should we sow the six species?

- This is a ternary diagram showing the predicted yield as the sown proportion of grasses, legumes and herbs is varied; and grasses, legumes and herbs here are represented by the G, the L and the H.
- At any point in this ternary diagram, the two species within those groups, within grass, legume and herb, the two species within those group are split in equal proportion.
- For example, at the vertex marked G for grass, this is the predicted yield for a 50:50 combination of the two grass species.
- And the closer a point is in the ternary diagram to that grass vertex, the higher the sown proportion of grass, and meaning of course as you move further away from that point, the sown proportion of grass is reducing.
- The pink point in the middle there is showing the predicted yield of the six-species multispecies mixture, where all species are sown in the same proportion.
- And the cyan point is showing the point at which we got the highest predicted yield.

SLIDE 8 – Comparison to high N grass?

- The predicted yield of our high N grass monoculture, that was 11.1 tonnes per hectare.
- But when does a multispecies mixture do better?
- We're going to ask the question – what proportions of grasses, legumes and herbs will lead to an outperformance, a higher performance, compared to the high N grass monoculture.
- So the second diagram on the right-hand side, is comparing each point on the predicted yield ternary diagram to the high N grass monoculture.
- Anywhere where you can see a blue region, this is a region where the multispecies mixture is outperforming the high N grass, and the lighter grey regions are where the multispecies mixture is comparable to the high N grass monoculture.
- This means you can change the proportions of grasses, legumes and herbs and still do better or comparably to the high N grass monoculture.
- The benefits of multispecies mixtures therefore were quite robust to changing the proportions of sown grasses, legumes and herbs.

SLIDE 9 – Comparison to 70:30 grass-legume?

- I'm going to ask the same question for the comparison to the one grass and one legume combination.
- The predicted yield for the ryegrass and white clover combination was 10.4 tonnes per hectare.
- And the result was quite similar, we found quite a wide range of sown proportions of grasses, legumes and herbs that outperformed the 70:30 grass-legume combination.

SLIDE 10 – What proportions?

- Essentially there is quite a wide region of sown proportions of grasses, legumes and herbs that will perform better than these conventional practices.
- The shaded region here highlights a wide gradient of sown proportions where the yield remained high.
- The six-species multispecies mixture with all six species equally sown is inside this region, but yields will remain high if for example, more legume is sown, or if the ratio of grass to herb is

changed. So this means that there is some flexibility in the sown proportions that can achieve high yields.

SLIDE 11 – Variation across sites?

- So far, we've looked at what happens on average across sites.
- Now, let's take a look at the raw data.
- Here you can see box plots representing the individual plot level yields at each site, with mixtures shown in the blue box plots and monocultures shown in the grey box plots.
- Generally you can see that the mixtures are higher than the monocultures at each site.
- And with our modelling approach, we showed that this difference was due to synergistic interactions, in particular, between species from the different groups, so from different groups across grass, legume and herbs.
- Here I've added in the average of the communities with the six species sown equally. So remember, that across our mixtures, we had a gradient from two up to six species.
- And generally the six-species mixture was among the better mixtures at each site.
- Here I've also added in the average of the high N grass monoculture plots, there shown with the red line.
- What you can see here is quite a lot of variation due to site location and a natural question arises here as to whether or not any of this variation across sites is due to climate.
- And we explored this through our modelling approach.

SLIDE 12 – Climate adaptation potential?

- What you can see here is predicted yield versus average daily temperature.
- On the temperature axis, you can see where the individual sites lie in the temperature gradient and where the average temperature is.
- The grey lines show the predicted yield for each of the six monocultures in the experiment.
- And the highlighted line, with the red triangles on it, that's showing the predicted yield for the high N grass monoculture across the temperature gradient.
- Now I've added in the 70:30 grass:legume predictions, again as they change across the temperature gradient.
- And what I'm going to add in next is the same prediction curve for the six-species mixture. You might wonder where this line will lie…
- As expected, it lies above high N grass and above the 70:30 grass:legume curves.
- But perhaps less, expected is that the difference between the six-species mixture curve and the conventional practices, that difference is actually increasing as the temperature increases.
- This is a crucial result in the context of the climate crisis. Temperatures are increasing across the globe, and we have shown that six-species multispecies mixtures have major potential to be used as a climate adaptation solution.
- This is excellent news for the future of productive grasslands!

SLIDE 13 – In summary

- In summary, our study focused in on multispecies mixtures.
- And we have shown that multispecies mixtures are better than one grass with higher nitrogen rates applied.

- We've shown that multispecies mixtures are better than a 70:30 perennial ryegrass, white clover combination.
- And we've shown that multispecies mixtures are robust to deviations in sowing proportions of grasses, legumes and herbs compared to sowing them all equally.
- And we've shown that multispecies mixtures are a promising climate adaptation solution.

SLIDE 14 – What next for LegacyNet?

- What next for LegacyNet?
- Well, you read the full story from the work I presented here in O'Malley et al, that's published in Science.
- We are also currently studying other agronomic outcomes such as weed invasion and forage quality.
- And in our experiment we had a follow-on crop sown in each plot after the grassland part of our experiment was completed. And we are currently investigating what are the effects of manipulating species diversity in a grassland ley on the performance of a follow-on crop.
- You can watch this space – there is plenty more to come from LegacyNet!
- Thank you very much.