Mass Spectrometry is a powerful tool to analyze different biomolecules in a tissue section. Both liquid chromatography-mass spectrometry (LC-MS) and mass spectrometry imaging (MSI) techniques are useful for large-scale detection and identification of small molecules, peptides, and proteins in samples, with MSI additionally being able to determine the spatial distribution of molecules within a tissue section. In this thesis, LC-MS and MSI approaches were applied to study symbiotic relationships. In the plant *Medicago truncatula*, a symbiotic relationship with rhizobia bacteria in specialized organs, root nodules, of the plant results in the reduction of atmospheric nitrogen into ammonia (termed biological nitrogen fixation). MSI methods were developed to investigate the small molecule and peptide content in the root nodules. Furthermore, a developed MSI method was applied to study salt stress, which negatively affects development of the symbiotic relationship in the root nodules. The other relationship explored was the complex interactions between the gut microbiome and its host. To investigate this complicated relationship, multiomics studies, which combine two or more single omics studies, such as metabolomics and proteomics, were utilized to achieve a more comprehensive analysis of the gut microbiome. Developed multiomic approaches were applied to study the response of the gut microbiome to pathogenic infection. Overall, this work develops a multifaceted approach to investigate symbiotic relationships, resulting in new method development, improved MSI sample preparation protocols, and increased knowledge about molecular players involved in the symbiosis.